

# Evaluation of certain veterinary drug residues in food

Eighty-first report of the Joint  
FAO/WHO Expert Committee on  
Food Additives



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W H O T e c h n i c a l R e p o r t S e r i e s  
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WHO Library Cataloguing-in-Publication Data

Evaluation of certain veterinary drug residues in food: eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives.

(WHO technical report series ; no. 997)

1.Food Contamination. 2.Drug Residues - analysis. 3.Drug Residues - toxicity. 4.Veterinary Drugs - toxicity. 5.Veterinary Drugs - pharmacology. 6.Risk Assessment. 7.Maximum Allowable Concentrations - standards. 8.No-Observed-Adverse-Effect Level. I.World Health Organization. II.Food and Agriculture Organization of the United Nations. III.Joint FAO/WHO Expert Committee on Food Additives. IV.Series.

ISBN 978 92 4 120997 7 (print)

ISBN 978 92 4 069550 4 (PDF)

ISSN 0512-3054

(NLM classification: WA 712)

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Design: Rania Spatha ([www.raniaspatha.com](http://www.raniaspatha.com))

Printed in Malta

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### Eighty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives

Rome, 17–26 November 2015

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<sup>1</sup> Attended session on dietary exposure assessment only.



## List of abbreviations

95/95 UTL	95/95 upper tolerance limit; upper limit of the one-sided 95% confidence interval over the 95th percentile of residue concentrations
ADI	acceptable daily intake
ARfD	acute reference dose
AUC	area under the concentration–time curve
AUC <sub>(0–72)</sub>	area under the concentration–time curve from 0 to 72 hours
BMD	benchmark dose
BMD <sub>10</sub>	benchmark dose for a 10% response over the controls
BMDL	lower 95% confidence limit on the benchmark dose
BMDL <sub>10</sub>	lower 95% confidence limit on the benchmark dose for a 10% response over the controls
BMR	benchmark response
bw	body weight
CAS	Chemical Abstracts Service
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
CI	confidence interval
CIFOCoss	Chronic Individual Food Consumption Database – Summary statistics
CL	clearance
C <sub>max</sub>	maximum concentration
CPU	4-chlorophenylurea
EDI	estimated daily intake
EHC	Environmental Health Criteria monograph
eq	equivalent
EU	European Union
F <sub>0</sub>	parental generation
F <sub>1</sub>	first filial generation
F <sub>2</sub>	second filial generation
FAO	Food and Agriculture Organization of the United Nations
GABA	gamma-aminobutyric acid
GDWQ	Guidelines for Drinking-water Quality (WHO)
GEADE	global estimate of acute dietary exposure
GECD	global estimate of chronic dietary exposure
GL36	Guideline 36 (VICH)
GLP	good laboratory practice
GVP	good practice in the use of veterinary drugs
H <sub>2</sub> B <sub>1a</sub>	22,23-dihydroavermectin B <sub>1a</sub> ' ; ivermectin B <sub>1a</sub>
H <sub>2</sub> B <sub>1b</sub>	22,23-dihydroavermectin B <sub>1b</sub> ' ; ivermectin B <sub>1b</sub>
HPLC	high-performance liquid chromatography

HPT	hypothalamic–pituitary–thyroid
INN	International Non-proprietary Name
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC <sub>50</sub>	median lethal concentration
LC-MS	liquid chromatography with mass spectrometry
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD <sub>50</sub>	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MIC <sub>50</sub>	minimum concentration required to inhibit the growth of 50% of organisms
MRL	maximum residue limit
MRT	mean residence time
MR:TR	marker residue to total residue ratio
MR:TRR	marker residue to total radioactive residue ratio
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organisation for Economic Co-operation and Development
PCA	4-chloroaniline; <i>p</i> -chloroaniline
ppb	parts per billion
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
rbSTs	recombinant bovine somatotropins
SD	standard deviation
$t_{\frac{1}{2}}$	half-life
T <sub>3</sub>	triiodothyronine
T <sub>4</sub>	thyroxine
T <sub>max</sub>	time to reach the maximum concentration ( $C_{max}$ )
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TRS	Technical Report Series
TSH	thyroid stimulating hormone
U	uniformly labelled
UGT	uridine diphosphate-glucuronosyltransferase
USA	United States of America
UTL	upper tolerance limit
UV	ultraviolet

VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
v/v	volume per volume
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme
WP	withdrawal period
w/v	weight per volume



Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

*Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 72, 2016.

Residue monographs are issued separately by FAO under the title:

*Residue evaluation of certain veterinary drugs.* FAO JECFA Monographs 18, 2016.

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# 1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Rome from 17 to 26 November 2015. The meeting was opened by Mr Ren Wang, Assistant Director-General of the Agriculture and Consumer Protection Department of the Food and Agriculture Organization of the United Nations (FAO), on behalf of the directors-general of FAO and the World Health Organization (WHO). Mr Wang noted that the Thirty-eighth Session of the Codex Alimentarius Commission has reconfirmed the fundamental role of JECFA in providing independent scientific advice on which Codex can base its deliberations. Furthermore, given the importance of the joint FAO/WHO activities on scientific advice related to food safety, both FAO and WHO have confirmed their continuing commitment and work in this area and are actively involved in discussions with Codex that will provide sustainable resources for the work of JECFA.

Mr Wang explained that the scientific advice that JECFA provides is a cornerstone in the process of providing guidance on food safety and ultimately ensures that food safety and quality measures and standards are based on sound scientific principles and ensure the protection of the health of consumers. Therefore, JECFA's work remains a high priority for both FAO and WHO.

Mr Wang reminded the Committee that participants have been invited to this meeting in their individual capacities as international experts and not as representatives of their organizations. He also reminded the Committee of the confidential nature of the meeting and stressed that the meeting report, which will need to be approved by the end of the meeting, will remain a restricted document until its publication is authorized by both FAO and WHO. Finally, Mr Wang expressed his sincere gratitude to participants for placing their valuable time and, most importantly, their expertise at the disposal of the two organizations and for the work that participants have already done in preparing to address the challenging agenda of the meeting.

Twenty meetings of the Committee had been held to consider veterinary drug residues in food ([Annex 1](#), references 80, 85, 91, 97, 104, 110, 113, 119, 125, 128, 134, 140, 146, 157, 163, 169, 181, 193, 208 and 217) in response to the recommendations of a Joint FAO/WHO Expert Consultation held in 1984 (1). The present meeting<sup>2</sup> was convened to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were:

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<sup>2</sup> As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives held in 1955 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been eighty previous meetings of JECFA ([Annex 1](#)).

- to elaborate further on principles for evaluating the safety of residues of veterinary drugs in food, for establishing acceptable daily intakes (ADIs) and acute reference doses (ARfDs) and for recommending maximum residue limits (MRLs) for such residues when the drugs under consideration are administered to food-producing animals in accordance with good practice in the use of veterinary drugs (GVP) (see [section 2](#));
- to evaluate the safety of residues of certain veterinary drugs (see [section 4](#) and [Annex 2](#)); and
- to respond to specific concerns raised by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) (see [sections 3](#) and [4](#) and [Annex 2](#)).

### 1.1 **Declarations of interests**

The Secretariat informed the Committee that all experts participating in the eighty-first meeting had completed declaration of interest forms. Professor Alan Boobis, Dr Kevin Greenlees and Dr Tong Zhou declared interests. As these interests were not related to topics on the agenda, they were not considered to be a conflict.

### 1.2 **Modification of the agenda**

The agenda (see [Annex 3](#)) was modified to exclude ethoxyquin, as no data were submitted by the sponsor.



## 2. General considerations

### 2.1 Matters of interest arising from previous sessions of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF)

The Codex Secretariat informed the Committee about relevant decisions of the Codex Alimentarius Commission and the principal outcomes and discussions of the Twenty-second Session of CCRVDF (2), which was held after the seventy-eighth meeting of the Committee in 2013 ([Annex 1](#), reference 217).

The Twenty-second Session of CCRVDF finalized work on the MRLs for derquantel, emamectin benzoate and monepantel that were recommended by the seventy-eighth meeting of the Committee; these MRLs were subsequently adopted by the Codex Alimentarius Commission at its Thirty-eighth Session (3). The Twenty-second Session of CCRVDF agreed to hold the MRLs for (1) ivermectin, because the recommended MRLs did not reflect approved GVP and in view of the request for re-evaluation that was put forward; and (2) lasalocid sodium, for which concern forms were submitted on the approach used to estimate short-term exposure of consumers and the possibility that the MRLs might expose consumers to residues higher than the ADI. The Committee noted that CCRVDF will consider the MRLs for lasalocid sodium at its next session (in October 2016) based on the Committee's recommendations. With regard to the MRL for lasalocid sodium in egg, CCRVDF established an electronic working group to prepare a discussion paper addressing the unintended presence of residues of veterinary drugs in food commodities resulting from carry-over of veterinary drugs into feed and to consider the establishment of MRLs at its next session as guided by the agreed policy.

The Twenty-second Session of CCRVDF finalized work on risk management recommendations for dimetridazole, ipronidazole, metronidazole and ronidazole, which were subsequently adopted by the Codex Alimentarius Commission at its Thirty-eighth Session. On the basis of the outcome of the evaluation of the seventy-eighth meeting of the Committee, CCRVDF had also formulated a risk management recommendation for gentian violet, which will be considered by the next session of CCRVDF.

The Codex Secretariat informed the Committee that the Twenty-second Session of CCRVDF had considered the outcome of the discussion by the seventy-eighth meeting of the Committee on the issue of MRLs in honey, including the key issues to be considered for recommending MRLs in honey. CCRVDF agreed to leave unaltered the current text of *Risk analysis principles applied by the Codex Committee on Residues of Veterinary Drugs in Foods* (4), noting that CCRVDF

can make requests to JECFA for MRLs for honey (and other commodities) using alternative approaches.

With regard to recombinant bovine somatotropins (rbSTs), the Twenty-second Session of CCRVDF considered the outcome of the JECFA re-evaluation, taking note of the report of the seventy-eighth meeting of the Committee. Although CCRVDF agreed that the Committee had addressed all of the questions posed to it by the Codex Alimentarius Commission, there were differing opinions regarding the Committee's replies. The Committee was informed that the Thirty-eighth Session of the Codex Alimentarius Commission recognized the validity of JECFA's risk assessments as the sound scientific basis for its deliberations on rbSTs. However, consensus was not reached on the adoption of the draft MRLs for rbSTs, and the Codex Alimentarius Commission agreed to hold the draft MRLs at Step 8 to provide further time to facilitate a consensus.

The Twenty-second Session of CCRVDF agreed on a priority list of veterinary drugs for evaluation (or re-evaluation) by JECFA and noted the importance of the commitment to submit the necessary data within the indicated time frame to ensure an efficient planning process for JECFA work.

The Twenty-second Session of CCRVDF requested the Committee to provide advice on the establishment of generic MRLs for fish species.

## 2.2 MRLs for generic fish species

The following two questions were forwarded to the eighty-first meeting of JECFA by the Twenty-second Session of CCRVDF (2).

*While recognising the ongoing activities of VICH [International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products] in this area, the Committee agreed to forward the following requests to JECFA:*

**1. To provide an assessment on whether on the basis of data from one or more fish species, it is possible to establish an MRL for finfish, crustaceans or molluscs in general, or for multiple similar groups.**

**Response from JECFA:** In 2012, the following question was posed to the seventy-eighth meeting of JECFA ([Annex 1](#), reference 217) by the Twentieth Session of CCRVDF (5):

Possibility of extending extrapolation by JECFA similar to that allowed under the current EU [European Union] guidelines. EHC [Environmental Health Criteria] 240 does not allow for the extrapolation of MRLs from muscle of Salmonidae to other finfish, but this

is allowable based on European Union guidelines. JECFA should consider extrapolation of MRLs between fish species. If the data required to support such MRL extrapolation is not available, what further work may be required?

The seventy-eighth meeting of JECFA responded:

JECFA must first receive information to confirm that there is an existing approval in a member state for use of the drug in the species of fish for which extrapolation of MRLs is requested, including a label or a statement of the approved conditions of use (GVP). The conditions of approved use (GVP) may differ depending on species of fish and region. However, the water temperature at which a product is used for treatment of fish and at which residue studies have been conducted are major considerations in the recommendation of MRLs for fish. This may result in different MRLs being recommended for different species, based on the GVP established for the use of the drug in one or more fish species in a member state or member states.

These concerns remain and are key factors in the JECFA evaluation of any substance for which data are provided for evaluation.

As of the seventy-eighth meeting of JECFA, only 10 substances had been evaluated by JECFA for the establishment of MRLs for finfish, and three of these substances were also evaluated for use in the treatment of crustaceans (shrimp). In most of these evaluations, the residue information reviewed by JECFA was primarily from the peer-reviewed scientific literature and reports from government laboratories and agencies.

No MRLs were recommended for four of these 10 substances (chloramphenicol, [Annex 1](#), reference 110; gentian violet, [Annex 1](#), reference 217; malachite green, [Annex 1](#), reference 193; and oxolinic acid, [Annex 1](#), reference 113) because an ADI could not be established by JECFA. Of the five substances for which JECFA has made recommendations of MRLs for finfish, two have been for “fish” and three for “salmon” and/or “trout”, based on the information provided. For the substances for which recommendations have been for “fish”, data have been provided for three or more diverse species of finfish.

Three substances have been evaluated by JECFA for use in the production of crustaceans; in all three cases, residue data provided were only for Giant prawn, also known as Black tiger shrimp (*Penaeus monodon*).

JECFA has not been requested by CCRVDF to recommend MRLs for any veterinary drug in any species of mollusc to date and also has not received any data regarding such use. Any comment on the feasibility of extrapolation of MRLs for mollusc species would therefore be speculative.

In conclusion, in order to properly address the issue of extrapolation of MRLs to fish species, JECFA requires, in addition to the information identified

by the seventy-eighth JECFA, further information on adequate groupings of fish species so that representative species can be identified from which MRLs may be extrapolated to other similar species. The Committee notes that several principles for grouping of fish species may be applied – for example, based on criteria such as a common aquaculture environment (salinity and temperature), phylogeny or common physiology (high lipid or low lipid) and common behaviour (demersal or not, type of diets). In any case, it would be necessary to develop clear boundaries around each group and define the inclusion and exclusion criteria for each group. JECFA is aware of work on this issue being conducted by VICH and will review the applicability of the guidance that results from that activity.

***2. For emamectin benzoate, to provide an assessment as to whether there are any identified toxicological, dietary exposure modelling, or analytical methodology issues preventing extrapolation of the proposed MRLs to a general finfish MRLs or a more appropriate sub-grouping.***

**Response from JECFA:** Although JECFA is not aware of any toxicological issues that would prevent extrapolation of MRLs to additional species of fish, the Committee has also noted, in response to a previous question on this issue from the Twenty-first Session of CCRVDF (6), that, in the absence of information, it is difficult to assure that there are no novel unknown metabolites of potential toxicological concern in tissues of the species for which no data have been available for evaluation.

The exposure modelling for emamectin benzoate was done by JECFA using data from depletion studies in Atlantic salmon. Median residues used in the exposure assessment could differ in other fish species, depending on the depletion profile, leading to higher or lower estimates of exposure. No information has been provided to JECFA to conduct such an assessment.

A primary issue in the consideration of the extension or extrapolation of the MRLs for emamectin benzoate residues in salmon and trout to additional species of fish is that the product containing emamectin benzoate intended for use in aquaculture for which information was provided to JECFA is intended only for treatment of sea lice in salmonids inhabiting cold water. Information is therefore required to demonstrate additional approved uses, and residue data are required to demonstrate the depletion profile in species other than salmonids; a suitably validated analytical method for any additional non-salmonid species would also be necessary.

In conclusion, in order to consider a request to extrapolate the MRLs recommended for salmon and trout to additional fish species, JECFA would require information on such approved uses, data to demonstrate pharmacokinetic and depletion behaviour of emamectin in a non-salmonid species and information to

demonstrate that the method validated for the analysis of the high lipid content tissue of salmon and trout is applicable to non-salmonid species, preferably a species with low lipid content.

### 2.3 Acute reference dose (ARfD) for veterinary drugs

Following a recommendation of the Committee at its seventy-fifth meeting ([Annex 1](#), reference 208), a working group to elaborate guidance on the establishment of ARfDs for veterinary drugs was formed. The working group developed a discussion paper, and key principles from this paper were discussed at the current meeting. The Committee agreed on the following principles, which will allow the working group to develop guidance on when and how to establish ARfDs for veterinary drugs:

- The main driver for the need to consider establishing an ARfD is the toxicological profile of the compound. For a veterinary drug, high exposure can also be a consideration.
- There are currently insufficient data to determine a generic toxicological cut-off value for acute effects based on exposure considerations; hence, the decision on whether to establish an ARfD is taken after consideration, case by case, of different (realistic) acute exposure scenarios, thereby allowing practical exposure considerations. As experience is gained, it may be possible to establish such a cut-off value, as has been done for pesticides.
- An appropriate acute dietary exposure assessment method needs to be used. The principles for a suitable method were described in EHC 240 (7), and details of the method were proposed in the report of the FAO/WHO workshop on dietary exposure to veterinary drugs (global estimate of acute dietary exposure, or GEADE) (8).
- The Committee clarified that the theoretical maximum daily intake (TMDI) calculation is a tool used as a proxy in dietary exposure assessment, in which a standard amount of food is combined with a selected highest residue level. The standard amounts of food used in the TMDI can be lower than the 97.5th percentile, as stated in EHC 240. Therefore, the TMDI is not appropriate for acute dietary exposure assessment.
- For establishing an ARfD for veterinary drugs, basic concepts as established for pesticide residues can be used. The key differences between veterinary drugs and pesticides relate to microbiological effects and to specific exposure scenarios. Regarding pharmacological effects – i.e. interaction with molecular targets (e.g. receptors) – it was

noted that this is not unique to veterinary drugs and that such effects do not automatically raise an acute health concern. Such effects need to be considered for acute and chronic health effects, in the same way as for toxic effects. In practice, this may lead to the same numeric value for the ADI and ARfD.

- Misuse (e.g. off-label use) of compounds is not within the scope of these considerations, just as they are not for chronic risk assessments.
- Regarding considerations for a microbiological ARfD, the Committee recognized that an acute exposure of the gut microbiota is different from the chronic daily exposure that JECFA evaluates to establish the microbiological ADI and that the most relevant microbiological end-point for acute exposure would most likely be disruption of the colonization barrier.
- It was noted that in extrapolating *in vitro* minimum concentration required to inhibit the growth of 50% of organisms ( $MIC_{50}$ ) values (and other microbiological data) to an effect dose *in vivo*, the factors to be considered differ from those used in establishing a microbiological ADI from such data. This could result in the incorporation of a different value for the correction factor used in the formula to calculate the microbiological ADI. The specific factor to be used would be compound specific, and guidance needs to be developed on the type of information necessary to enable the Committee to estimate such a factor. Consideration will also need to be given as to what would be an appropriate default factor in the absence of compound-specific information.
- When discussing the implications for MRL recommendations, the Committee suggested to continue with MRL derivations that are compatible with chronic exposure (i.e. the ADI) and the respective withdrawal times. If an ARfD is established for the compound as well, an acute exposure assessment will then be performed based on tissue concentrations at the estimated withdrawal times, and the consequences will be described in detail. If the ARfD is exceeded, this will be reported, and possible refinements of the assessment will be described, including options such as the selection of a later time point for the recommendation of MRLs.

The working group will continue its work on a draft guidance, which will be made available for public comments before further discussion at the next JECFA meeting dealing with veterinary drug residues in food.

The Committee recommended that a subgroup be established to review available information on acute exposure to residues of veterinary drugs and

to identify an upper-bound exposure value with sufficient confidence that will enable, if possible, the derivation of a cut-off value for acute toxicity.

## 2.4 Chronic dietary exposure assessment

During its previous meetings, JECFA agreed to develop an approach to assess more accurately the chronic dietary exposure to veterinary drug residues (global estimate of chronic dietary exposure, or GECDE). At the present meeting, the Committee decided to continue using this approach in parallel with the estimated daily intake (EDI) model in order to gain experience and to continue improving the methodology. Moreover, the Committee identified two additional important issues concerned with the methodologies applied by JECFA and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) to estimate chronic dietary exposures that merit general consideration.

### 2.4.1 Approach for dietary exposure assessment of compounds used for multiple purposes (i.e. veterinary drugs and pesticides)

As a consequence of its consideration of two veterinary drugs (teflubenzuron, see [section 4.4](#); and diflubenzuron, see [section 4.1](#)) at the present meeting, the Committee identified the issue of how to estimate chronic dietary exposure to residues of substances used as both veterinary drugs and pesticides. The Committee noted that it has been common practice to assess the chronic exposure of pesticide and veterinary drug residues using different approaches that have been developed after consideration of the types of substances of interest, duration of exposure, exposure in different subgroups and the type of estimate needed, based on the information available. However, the Committee expressed the view that it may be necessary to estimate the total chronic exposure from both sources.

The Committee noted a number of compounds that have been evaluated by JECFA as well as by JMPR: abamectin, cypermethrin and alpha-cypermethrin, cyfluthrin, cyhalothrin, deltamethrin, diflubenzuron, emamectin benzoate, thiabendazole and teflubenzuron.

The Committee identified some possible approaches to estimating the total chronic exposure to residues from these compounds. The easiest approach would be to sum up the chronic exposure estimates derived by the two expert committees to arrive at an estimate of total chronic exposure. Alternatively, the JMPR or JECFA methodologies could be extended to estimate chronic exposure from all sources. Finally, the most comprehensive approach would be to develop a specific chronic exposure model that would fit both JMPR and JECFA risk assessment purposes.

The Committee noted that simply combining chronic exposure estimates or hybridizing methodologies would mix estimates underpinned by



different assumptions about chronic consumption, one using average per capita consumption (JMPR) and the other using model diets that aim to cover high-percentile consumption in any population group (JECFA). The Committee was of the opinion that such an approach may be used in the interim, but might not be rigorous enough in the longer term.

The Committee saw merit in developing a comprehensive solution to the challenge of chronic exposure assessment of residues of substances used as veterinary drugs and pesticides by developing a new methodology for estimating exposure that would suit JMPR and JECFA risk assessment purposes. However, the Committee noted that it would take some time to develop, implement and validate such a method.

In conclusion, the development of chronic dietary exposure assessment methods that take into account combined chronic exposure from pesticide and veterinary drug residues should be investigated. These methods should be robust and fit both JMPR and JECFA risk assessment purposes.

#### 2.4.2 Dietary exposure assessment for less-than-lifetime exposure

Based on the consideration raised by JMPR, the Committee noted that the current long-term chronic dietary risk assessment of pesticides is based on multi-annual consumption data averaged over the whole population to capture the per capita dietary pattern over a lifetime. However, no-observed-adverse-effect levels (NOAELs) for pesticides derived from animal studies with exposure ranging from 4 weeks to 104 weeks are often similar. This suggests that over wide exposure duration ranges, adverse effects generally are not related to duration of exposure. JMPR considered that in consequence, short-term (weeks to months) or consumer-only exposures may not be adequately described to determine whether an ADI is exceeded in these situations and whether this would result in health concerns. The present Committee noted that its chronic dietary exposure model (GECDE) is addressing the exposure of consumers over times shorter than a lifetime.

However, the Committee noted that there are examples of veterinary drugs where the duration of exposure was an important consideration in the toxicological evaluation (e.g. sisapronil, this meeting; see [section 4.3](#)). The frequency with which the toxicity of veterinary drugs progresses after exposures of 4 weeks is unknown and should be evaluated. Based on the outcome of this exercise, it may be necessary to align the choice of the dietary exposure model with duration of exposure at which the adverse effects occur.



### 2.4.3 Recommendations

The Committee recommends that the FAO and WHO Secretariats convene an expert meeting on two important issues concerned with the methodologies applied by JECFA and JMPR to estimate chronic dietary exposures.

*In regards to dietary exposure assessment of compounds used for multiple purposes (i.e. veterinary drugs and pesticides):*

1. Develop chronic dietary exposure assessment methods that take into account combined exposure from pesticide and veterinary drug residues.
2. Investigate the applicability of these methods using compounds that have been evaluated as both pesticides and veterinary drugs.

*In regards to dietary exposure assessment for less-than-lifetime exposure:*

1. Investigate the effects of duration of exposure in toxicity studies on veterinary drugs on toxicological end-points and the points of departure (e.g. NOAELs).
2. Based on the outcome of #1, identify those toxicological situations requiring less-than-lifetime exposure assessment.

*In regards to dietary exposure assessment*

1. Apply the methodologies developed above to some key examples of veterinary drugs and pesticides that are unlikely to accumulate (including compounds that have been evaluated as both pesticides and veterinary drugs) and report the outcome to JECFA and JMPR.

## 2.5 Update and revision of *Principles and methods for the risk assessment of chemicals in food* (EHC 240)

The Committee discussed ways in which exposure from the dual use of a substance as both a pesticide and a veterinary drug might be assessed. It was recommended that a joint JECFA (veterinary drug residues)/JMPR multidisciplinary working group be established to develop suitable methodology (see [section 2.4.1](#)). In addition, this group should consider the recommendations of the 2015 JMPR (9) regarding shorter-than-lifetime exposure (see [section 2.4.2](#)). Depending on the outcome of this exercise, the relevant section(s) of EHC 240 should be updated.

The Committee was updated on and discussed the key issues in the development of guidance for the establishment of ARfDs for residues of veterinary drugs. This poses some unique challenges, such as the possibility of acute antimicrobial effects. The working group developing this guidance will complete its draft guidance and submit it for public comment before placing it on the agenda for the next JECFA (veterinary drug residues) meeting (see [section 2.3](#)). Once finalized, this will necessitate suitable addition to EHC 240.

The Committee discussed whether processing data should be sought for all residues of veterinary drugs. It was agreed that this would not be practical, but that the issue should be dealt with on a case-by-case basis, where there was some reason for possible concern (see [section 2.8](#)). Some minor amendment of EHC 240 might be necessary to reflect this.

The Committee agreed to adopt the practice of JMPR to consider identifying an overall NOAEL for studies in dogs of 90 days' and 12 months' duration (see [section 2.10](#)). EHC 240 should be updated to reflect this procedure, now in use by both JMPR and JECFA (veterinary drug residues).

## 2.6 **Guidance for the evaluation of veterinary drug residues in food by JECFA**

The Committee was provided with drafts of the revised guidance documents for JECFA monographers and reviewers evaluating residues of veterinary drugs. While these guidance documents are intended primarily for JECFA Experts who prepare residue and toxicological monographs for JECFA and for Members (reviewers) who have been assigned to peer review them and propose evaluations, they will also be useful to manufacturers who submit dossiers to JECFA and other parties interested in understanding the process followed in the evaluation of residues of veterinary drugs in food by JECFA.

The Committee was asked to provide written comments to the respective Secretariat by the end of 2015 so that the documents can be finalized early in 2016.

## 2.7 **Update on FAO and WHO databases related to the work of the Committee**

The current FAO JECFA databases (one for food additives, one for flavouring agents and one for residues of veterinary drugs) were developed in early 2000 and are based on outdated underlying software. The FAO Secretariat has therefore started a project to modernize the three databases.

Although the major features and output will not differ significantly from the current version, the project aims to develop an online platform that allows

the Secretariat to manage the process from adding records to or updating records in the database to publishing the adopted JECFA evaluations. The new databases will also allow for improved interconnectivity with other databases, such as the Codex database of adopted MRLs of residues of veterinary drugs and the WHO summaries of JECFA evaluations.

The new databases are currently being finalized and should be operational in the next months.

To improve the data used for dietary exposure assessment, FAO and WHO continue to collect and compile national individual food consumption data. Summary statistics from (currently) 37 surveys (only those with a duration of 2 days or more) from 26 countries are published in the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCos).

## 2.8 Processing of food containing residues of veterinary drugs

During the evaluation of diflubenzuron by the present Committee (see [section 4.1](#)), the issue of its thermal degradation to 4-chloroaniline (*p*-chloroaniline or PCA), a metabolite of substantial toxicological concern, was discussed. As this reaction can occur at temperatures readily achieved during home cooking (>100 °C), this had to be taken into account in the risk assessment of the residues of diflubenzuron. In the evaluation of residues of pesticides by JMPR, the effect of processing, including cooking in the home, on the amount and nature of the residues ingested by consumers is routinely considered. The present Committee therefore considered whether this should also be undertaken routinely in its assessment of residues of veterinary drugs.

The Committee noted that whereas for many pesticides, residue levels may be reduced or eliminated prior to cooking (e.g. residues in skin would be removed by peeling), this would rarely, if ever, be the situation for residues of veterinary drugs. In addition, the variation in cooking conditions and temperatures of food containing residues of veterinary drugs is appreciably greater than that for food containing pesticide residues, as would be the impact on the bioavailability of non-extractable residues. Also, more foods containing pesticide residues are eaten raw (without cooking) than are foods containing residues of veterinary drugs. These factors would make the task of routinely assessing the effects of processing of foods on residues of veterinary drugs much more complex and onerous than when assessing pesticide residues. Reflecting this, such information is not routinely requested by regulatory authorities (e.g. European Medicines Agency, United States Food and Drug Administration) involved in the assessment of veterinary drugs for use in food-producing animals.

The Committee therefore concluded that it would not routinely assess, or seek to address, the effects of processing foods on residues of veterinary drugs.

However, if there is evidence, or some other reason to suspect, that processing of foods containing residues of specific veterinary drugs could have toxicological implications, such as for diflubenzuron, the effect of processing should be taken into consideration in the assessment of that compound.

## 2.9 Reporting of original data in JECFA monographs

JECFA publishes its assessments of residues of veterinary drugs in food in the form of monographs (toxicological evaluations in the WHO Food Additives Series and residue evaluations in FAO JECFA Monographs) and summaries in the form of reports in the WHO Technical Report Series. Although the Committee seeks to be as transparent as possible in these publications, JECFA, like other organizations involved in the evaluation of veterinary drugs, is sometimes constrained, by requirements for confidentiality, in the information that it can make available publicly. However, subject to this restriction, in reporting its findings, the Committee will seek to publish such information as necessary to enable the basis of its conclusions to be clearly understood and independently verified.

## 2.10 Assessment of short-term (90-day and 12-month) studies in dogs

Following analysis of a number of databases comprising information from several hundred compounds, including many pesticides, many authorities (including the United States Environmental Protection Agency, European Commission and JMPR) concluded that the nature and potency of effects observed after oral administration to dogs for 90 days rarely showed any change after a further 9 months of administration; in other words, the effects and the NOAELs at 12 months were the same as at 90 days. As a consequence, it was recommended that there was need for only a 90-day study in dogs, and this has since been reflected in the Organisation for Economic Co-operation and Development (OECD) test guideline for short-term studies in dogs.

JMPR noted that in light of this, it would be possible to consider most 90-day and 12-month studies in dogs to be short-term repeated-dose studies providing the same information. Hence, following the same considerations as for two studies of the same duration (see JECFA guidance (10)), it would be possible to identify an overall NOAEL (and lowest-observed-adverse-effect level, or LOAEL) for the studies. It was agreed that JECFA would adopt the same practice and that the guidance should be amended accordingly.

## 2.11 Coordination of the agendas of JECFA and JMPR

JMPR evaluates residues of pesticides in food, whereas JECFA (veterinary drug residues) evaluates residues of veterinary drugs in food. In general, although there are many assessment principles in common – and these are being harmonized to the extent possible – the groups tend to operate largely independently.

There are some substances that are used both as pesticides and as veterinary drugs – for example, teflubenzuron at the present meeting (see [section 4.4](#)). Because of differences in their residue profiles and exposures when used, respectively, as a pesticide and a veterinary drug, both JMPR and JECFA will be asked to assess such compounds for both their toxicology and their residues. In general, different experts are involved in the assessment of the compounds by JECFA and JMPR, and hence it is quite possible that there will be some differences in the interpretation of data and the conclusions reached. It is also possible that there are different sponsors for the substance when used as a pesticide and when used as a veterinary drug, which could lead to differences in the data made available to the respective experts. Indeed, this might even happen when the sponsor is the same, but different departments are responsible for pesticide and veterinary use. In the event that this leads to different outcomes or recommendations – for example, the ADI established – this would lead to confusion among those relying on such assessments.

Hence, the present Committee strongly recommends that where dual-use substances are to be evaluated by both JMPR and JECFA, the Codex Committee on Pesticide Residues (CCPR) and CCRVDF coordinate the prioritization of such substances for evaluation by the respective experts. The Committee also recommends that the Joint Secretariats of JMPR and JECFA ensure that there is suitable interaction between experts in the evaluation of such compounds.



## 3. Response to concern forms from CCRVDF

### 3.1 Lasalocid sodium

#### *Background*

The seventy-eighth meeting of JECFA ([Annex 1](#), reference 217) evaluated lasalocid sodium and established a microbiological ADI with an upper bound of 8.4 µg/kg body weight (bw) or 504 µg/person, applying the standard approach of VICH Guideline 36 (GL36), for chronic exposure to lasalocid residues. The toxicological ADI, with an upper bound of 5 µg/kg bw or 300 µg/person, based on effects observed in developmental and reproductive toxicity studies in experimental animals, was lower than the microbiological ADI and thus was considered to be more relevant in the assessment of lasalocid sodium. The seventy-eighth Committee recommended MRLs that are compatible with this ADI, using the EDI approach for chronic exposure assessment. At the Twenty-second Session of CCRVDF (2), the Delegation of the EU expressed concern regarding a potential for acute health risk from intake of lasalocid residues and submitted a concern form. No new data were provided with the concern form.

#### *Concern from EU*

The EU concern read as follows:

In the case of lasalocid sodium, JECFA identified a hazard that may occur following short term exposure to residues: a disruption of the colonisation barrier. The level of consumer exposure that JECFA considered not leading to a disruption of the colonisation [barrier] was expressed as a microbiological ADI (8.4 µg/kg bw or 504 µg/person). Because this hazard may occur following a short term residue exposure, there must be assurance that even occasional, high residue intake will not exceed the microbiological ADI. The EDI cannot provide this assurance. JECFA is developing a complementary approach for addressing short term exposure scenarios based on high residue intake. However, this work has not yet been finalised. Therefore JECFA was unable to assess this kind of exposure. If the TMDI approach is used for this purpose – which is the approach that JECFA has used in other cases where short term exposure may lead to a consumer safety concern – the proposed draft Codex MRLs for poultry tissues would be estimated to lead to a consumer exposure of 882.11 µg/person, which represents 175% of the JECFA microbiological ADI thus representing a risk to consumer health.

**Response from JECFA:** The EU has raised a concern with respect to short-term exposure, interpreted by the present Committee to mean acute exposure, to lasalocid residues and the potential disruption of the colonization barrier

in the gastrointestinal tract. In the concern raised, it is assumed that the upper bound of the microbiological ADI is the appropriate reference point for acute microbiological effects. The conclusion is provided that the estimated exposure, using the TMDI approach as a proxy for acute exposure, would lead to an exceedance of the microbiological ADI and therefore represents a health concern.

The Committee recognizes that when antimicrobials are used at high doses in humans, this might acutely affect the intestinal microbiota. However, the Committee concludes that the upper-bound microbiological ADI of 8.4 µg/kg bw or 504 µg/person for lasalocid sodium established at the seventy-eighth meeting of JECFA is protective of the health of the consumer and that significant disruption of the colonization barrier would not occur even at acute exposure levels.

The microbiological ADI established by the seventy-eighth meeting of JECFA was derived in part from MIC<sub>50</sub> data on relevant human intestinal microbiota. The Committee does not consider that the microbiological ADI derived from such data represents an appropriate health-based guidance value with which to assess the risks of effects on the intestinal microbiota from acute exposure to residues of lasalocid. The reasons for this are outlined below.

There are differences in exposure of the intestinal microbiota in the colon following acute and chronic oral doses of the veterinary drug residue in food.

The concentration of a lasalocid residue following a single dose would initially enter the oesophageal/gastrointestinal tract as a one-time bolus in a meal and then be subjected to dilution, binding and gastric emptying processes while traversing the intestinal tract to the colon, which has not previously been exposed to lasalocid. In contrast, chronic daily ingestion of the same amount of lasalocid residue each day for a lifetime, as assumed in the microbiological ADI calculation, would result in drug residues continuously transiting through the gastrointestinal tract, with the potential for lasalocid to accumulate, resulting in an increased concentration in the colon, and impact the intestinal microbiota on a daily basis. Thus, the residue concentrations to which intestinal bacteria are exposed following an acute dose will be lower than those occurring following chronic ingestion at the upper-bound microbiological ADI; unlike the situation with chronic exposure, there would be no possibility of an additive effect over time. From the above considerations, it is reasonable to assume that the fraction of the oral dose of lasalocid available to intestinal bacteria would be lower following acute exposure than following chronic daily exposure to the same amount of drug residue each day for a lifetime.

It is important to note that an observed minimum inhibitory concentration (MIC) value (i.e. bacterial growth inhibition) is an *in vitro* measure of effect, but one cannot translate this directly to an acute *in vivo* level of bacterial exposure to the drug in the intestinal tract. The MIC standard test methodology, while



a well-accepted in vitro measure of bacterial susceptibility to an antibacterial drug, does not take into account the interactions of the intact gastrointestinal environment – such as the complexity of the intestinal microbial community, drug binding with food components or intestinal contents, metabolism by the host and the resilience of the intestinal microbiota to a single versus repeated exposure – and thus overestimates the drug potency in vivo. Therefore, the MIC test itself does not provide a measure of the concentration that would produce an acute effect in vivo. The result is a conservative estimate of the microbiological ADI when applied to chronic effects and an overly conservative estimate when applied to acute effects on the human intestinal microbiome. It should also be noted that the formula given in EHC 240 (also presented in VICH GL36) that is used in the assessment of lasalocid results in a conservatively low estimate of the upper-bound microbiological ADI, as the MIC calculation utilizes the lower 90% confidence limit of the MIC<sub>50</sub> of those groups of bacteria against which lasalocid is active and does not take into account the MICs of those groups of intestinal microbiota that are not sensitive.

Based on the data that the Committee reviewed at the seventy-eighth meeting, faecal binding of lasalocid was greater than 90%, a value that was used in deriving the microbiological ADI. When considering acute exposure, drug binding may be even higher than following chronic exposure because of the greater number of luminal binding sites available to the drug, and hence the fraction of a single dose of lasalocid available to microorganisms might be lower under conditions of acute exposure to the veterinary drug residues compared with chronic exposure to those residues. It should also be noted that the use of a mass of colon content of 220 g in the formula is a conservatively low estimate, as recent data indicate that the colon volume is, in fact, much higher than this (561 mL).

The Committee is continuing to work on the implementation of its global approach to assessing the acute risk from ingestion of residues of veterinary drugs, including microbiological risk, when relevant. However, it should be emphasized that with respect to the assessment of acute dietary exposure (to be compared with an ARfD), the methodology is already fully described in the report of the FAO/WHO workshop on dietary exposure to veterinary drugs (8).

The Committee wishes to clarify that JECFA does not use the TMDI approach for assessing acute dietary exposure for risk assessment and that the TMDI is not appropriate for this purpose. The TMDI calculation is a tool used as a proxy in dietary exposure assessment, in which a standard amount of food is combined with a selected highest residue level. The standard amounts used in the TMDI can be lower than the 97.5th percentile, as stated in EHC 240. The Committee does not consider the microbiological ADI to be an appropriate health-based guidance value for conducting an acute risk assessment. Although the Committee has not finalized its methodology for establishing a microbiological

ARfD, as a conservative surrogate, it used the upper-bound microbiological ADI after taking into account some factors that will impact on this value. As discussed above, recent data support a colon volume of 561 mL (g), rather than 220 g (mL). In addition, whereas a value of 90% was used for faecal binding of lasalocid by the seventy-eighth JECFA, this was a very conservative estimate, and a more realistic estimate, based on information provided to the seventy-eighth JECFA, would be 95–99%, which might be further increased with an acute exposure. Assuming binding of 95%, together these factors would result in an increase in the upper-bound microbiological ADI by 5.0-fold. The extent of dilution of the microbiologically active residues following an acute exposure and the resilience of the gut microbiota to an acute exposure will further increase this value, but the Committee did not have sufficient information to quantify the impact of these factors at this time.

The Committee estimated acute exposure to lasalocid residues using the methodology developed for this purpose (GEADE) and concluded that it would be approximately 3.5-fold higher than the upper bound of the microbiological ADI established by the seventy-eighth JECFA. Taking into consideration the possible acute microbiological effect (>5-fold higher than the upper bound of the microbiological ADI) and acute exposure to lasalocid residues (not greater than 3.5-fold higher than the upper bound of the microbiological ADI), the Committee concluded that there would be no concern for colonization barrier disruption in the colon from acute exposure to residues of lasalocid.

The Committee recognizes the need to develop an approach for establishing a microbiological ARfD, as it is possible that the acute ingestion of an antimicrobial veterinary drug could affect the colonic microbiota, but there are a number of important differences in how an acute exposure to microbiologically active residues should be evaluated compared with those ingested chronically. Consideration also needs to be given to the data that would be necessary to enable the establishment of a microbiological ARfD. These issues are currently under discussion by the working group that is developing guidance on establishing an ARfD for veterinary drugs (see [section 2.3](#)).

### **Concerns from Canada**

The concerns raised by Canada were presented in RVDF/22 CRD 27, Agenda Item 6(c), at the Twenty-second Session of CCRVDF (2), with the subsequent submission of a concern form:

Canada would like to raise the following scientific points for further consideration by JECFA:

1. The MRLs proposed for this compound were calculated based on the estimated daily intake (EDI) approach. Canada had earlier expressed the concern that there would be limitations with using the EDI approach when residue depletion data are highly variable. In the case for lasalocid residues in chicken tissues (see Table 7.5 of the monograph) the standard deviations of residues in each tissue on 1-day withdrawal period (WP) (time for which exposure estimates were evaluated) were much higher than the mean of the residues (i.e., the coefficient of variation was > 100%). Mean and standard deviations of lasalocid A residues at 1-day WP were respectively, 65 ppb [parts per billion] and 103 ppb in muscle, 244 ppb and 329 ppb in liver, 128 ppb and 194 ppb in kidney, and 106 ppb and 165 ppb in skin/fat of chickens. Given the highly variable nature of the data used to derive the MRLs, Canada considers that this approach may not be robust enough for the establishment of lasalocid MRLs in order to ensure safety to consumers.

**Response from JECFA:** The Committee has considered the concern expressed by the Member State. In developing the EDI procedure, the sixty-sixth meeting of the Committee ([Annex 1](#), reference 181) concluded that “the TMDI was no longer the most suitable estimate of chronic intake, because the MRL was a single concentration representing the estimated upper limit of a high percentile of the distribution of marker residue present in a given tissue of the treated animals”. The sixty-sixth meeting of the Committee concluded that

it was not realistic to use an extreme value of the distribution in a scenario describing chronic intakes. In such a scenario, all concentrations of the distribution of residues should be considered. The median concentration represents the best point estimate of a central tendency over a prolonged period of time, because the concentration of residues in a given tissue consumed varies from day to day, as reflected in the distribution. Therefore, the Committee decided to use the median of the residue distribution to substitute for the MRL in the intake estimate.

While acknowledging that the lasalocid data are variable, the current Committee noted that the EDI approach has been applied in other assessments where residue data were variable. Additionally, the Committee noted that the median is not unduly affected by outliers. Finally, the Committee noted that variability in residue values is not uncommon in studies involving poultry or when dosing via feed. The observed variability associated with lasalocid residue values does not appear to be the result of a systematic bias. The current Committee concluded that the lasalocid residue depletion data are robust, were collected in a good laboratory practice (GLP)–compliant study and can be used with the EDI approach.

2. JECFA monograph indicates that the residue data from 1-day WP was used to derive the proposed MRLs. However, marker to total residue (MR:TR) ratios based on data for 0-day WP were used instead. There is significant reduction in MR:TR between the 0-day and 1-day WP (see Appendix below). After 1-day WP, the MR:TR remains fairly stable. Hence, the MR:TR ratio at 0-day would likely under-estimate the total exposure. Canada therefore considers that MR:TR based on 1-day WP of <25% for muscle, 8.8% for liver, 14.2% for kidney and 29.2% for skin/fat (see Table 7.2 of the monograph) should perhaps be used along with the residue depletion data in the exposure assessment.

**Response from JECFA:** As noted in the monograph prepared for the seventy-eighth JECFA (Table 7.2, footnote; [Annex 1](#), reference 218), the withdrawal times for the radiolabelled residue depletion study are actually 16 hours post last dose relative to their designation (i.e. “0” withdrawal is actually 16 hours post last dose). For the current assessment, all the withdrawal times are restated to clearly indicate the elapsed time from the final dosing. Following this re-presentation of the withdrawal times in the radiolabelled residue depletion study, it is clear that the withdrawal times in that study align more closely with the withdrawal times in the residue depletion study using non-radiolabelled drug than was apparent from Table 7.2. The marker residue to total radioactive residue (MR:TRR) ratios at 16 hours post last dose are 55% (muscle), 52% (skin/fat), 22% (liver) and 41% (kidney).

Using a different approach, interpolated MR:TRR values were developed. For muscle, where there was no MR:TRR at 40 hours post last dose (formerly designated 24 hours withdrawal), the hypothetical 25% MR:TRR for muscle proposed by the requestor was used. The formula  $(MR:TRR_{16} - MR:TRR_{40})/3$  was used to calculate the change-over-time in the MR:TRR ratio between 16 and 40 hours post last dose in 8-hour increments, and this value was then subtracted from  $MR:TRR_{16}$  to give  $MR:TRR_{24}$ . The interpolated MR:TRR ratios at 24 hours post last dose are 45% (muscle), 44% (skin/fat), 18% (liver) and 32% (kidney).

Using either the experimentally derived MR:TRR ratios or those MR:TRR ratios developed through the interpolation, both the EDI and the GECDE remain below the ADI for adults, children and infants. However, because the re-presented sample collection times in the radiolabelled residue depletion study align well with the sampling times in the depletion study using unlabelled drug, the experimentally derived MR:TRR ratios at 16 hours post last dose are used in conjunction with MRLs derived from the 1-day withdrawal residues in the residue depletion study using unlabelled drug in the exposure assessment for lasalocid in chicken tissues.

3. When the data are insufficient or of quality not suitable for the EDI approach, the JECFA has historically used the theoretical maximum daily intake (TMDI) approach to establish MRLs. Based on our calculation using the same data but using the TMDI approach, if the exposure was estimated using the proposed MRLs and the marker to total residue ratios at 1-day WP, the daily human exposure to lasalocid residues would be 2157.6 µg per person which is 7 times higher than the ADI value of 300 µg per person (see Table 6 of Appendix for detailed calculation).

**Response from JECFA:** The Committee has concluded that when data are sufficiently robust to support the use of the EDI approach, that approach will be used, because it is more representative of actual exposure from the consumption of tissues derived from treated animals. The lasalocid residue depletion data are robust, were collected in a GLP-compliant study and can be used with the EDI approach (see also the response to #4).

4. While Canada understands that the new dietary exposure assessment approach piloted by the JECFA in its 78th meeting is still being verified, the global estimate of chronic dietary exposure (GECDE) using the MR:TR on 1-day WP for lasalocid would have exceeded the ADI. The GECDE represents 92% of ADI for adults, 168% of ADI for children and 149% of ADI for infants (see Appendix for calculations). JECFA's conclusion that the GECDE is below the ADI was because of considering the MR:TR for 0-day WP which we believe underestimates the exposure. Given that 1-day WP residue data does not support the safety to consumers based on GECDE approach, perhaps the residue data from 2-day WP would have been ideal to establish MRLs for this compound. The 95th percentile (upper 95% CI [confidence interval]) of residue data at 2-day WP would have yielded the MRLs of 100 ppb in muscle, 500 ppb in liver, 250 ppb in kidney and 200 ppb in skin and fat (see Appendix, Table 7).

**Response from JECFA:** Following re-presentation of the sampling times in the radiolabelled residue depletion study to clearly reflect the actual time post last dose at which the samples were collected, it is clear that the sampling times in that study and the sampling times in the residue depletion study using non-radiolabelled drug align well and can be used to derive MRLs for the use of lasalocid in chickens. Using the MR:TRR at 16 hours post last dose, both the EDI and the GECDE remain below the upper bound of the ADI for adults, children and infants.

An EDI of 80 µg/person per day was calculated, based on median residues for a 1-day withdrawal in chicken, which represents 27% of the upper bound of the ADI, based on a 60 kg individual.

The GECDE for the general population is 1.85 µg/kg bw per day, which represents 37% of the upper bound of the ADI. The GECDE for children is 3.38 µg/kg bw per day, which represents 67% of the upper bound of the ADI. The GECDE for infants is 2.99 µg/kg bw per day, which represents 60% of the upper bound of the ADI.

In addition to the numbered questions, the Member State raised the additional concern that they were not able to reproduce the results contained in Table 7.2 of the JECFA monograph.

**Response from JECFA:** The values in Table 7.2 of the JECFA monograph are correctly calculated. For complete transparency, the individual residue values for each animal and each tissue assayed in both the radiolabelled residue depletion study and the residue depletion study using unlabelled drug are presented in the current addendum to the residue monograph.

### **Conclusions**

Following consideration of the issues raised in the concern forms, the ADI established and MRLs recommended at the seventy-eighth meeting of JECFA remain unchanged.

## 4. Comments on residues of specific veterinary drugs

The Committee evaluated or re-evaluated five veterinary drugs. Information on the safety evaluations is summarized in [Annex 2](#).

### 4.1 Diflubenzuron

#### *Explanation*

Diflubenzuron (International Union of Pure and Applied Chemistry [IUPAC] name: 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea; Chemical Abstracts Service [CAS] no. 35367-38-5) is an acyl urea derivative (halogenated benzoylphenylurea). Diflubenzuron is approved for use as a veterinary drug in Norway and Chile in the treatment of sea lice (*Lepeophtheirus salmonis* and *Caligus rogercresseyi*) infestations in Atlantic salmon (*Salmo salar*) as an oral dosage of 3–6 mg/kg bw in feed for 14 consecutive days, with a withdrawal period in the range of 105–300 degree-days. It is also used as an insecticide/acaricide in agriculture and forestry against larvae of Lepidoptera, Coleoptera, Diptera and Hymenoptera and as a vector control agent in drinking-water sources and drinking-water storage containers.

The mechanism of action of diflubenzuron is to inhibit the formation of new chitin in the insect cuticle during the moulting process by inducing both chitinase and phenoloxidase.

Diflubenzuron has not previously been evaluated by the Committee. The Committee evaluated diflubenzuron at the current meeting at the request of the Twenty-second Session of CCRVDF (2). The Committee was asked to establish an ADI and recommend MRLs for diflubenzuron in salmon muscle and skin in natural proportion.

The Committee noted that the toxicity of diflubenzuron has been previously evaluated by JMPR in 1981, 1985 and 2001 (11–13) and by a number of other scientific or regulatory bodies, including the WHO Task Group on Environmental Health Criteria for Diflubenzuron, which prepared Environmental Health Criteria 184 (14). In 2001, JMPR established an ADI of 0–0.02 mg/kg bw for diflubenzuron, based on the NOAEL of 2 mg/kg bw per day for haematological effects observed in 2-year toxicity studies in rats and a 52-week toxicity study in dogs (13).

The metabolism of diflubenzuron is known to lead to the formation of PCA in some species, but not the rat, and it is not known whether PCA is formed in salmon or humans. PCA might also occur as an impurity in the product formulation or as a degradation product generated during food processing. PCA is considered by many scientific and regulatory bodies (e.g. International

Agency for Research on Cancer) as genotoxic and carcinogenic. The Committee therefore evaluated the toxicity of PCA, focusing particularly on its genotoxicity and carcinogenicity as well as on its possible carcinogenic mode of action, based on studies retrieved from a search of published literature.

### ***Toxicological and microbiological evaluation***

Because the sponsor did not provide any toxicological data on diflubenzuron, the Committee relied mainly on the summary evaluation prepared by JMPR in 2001. The Committee considered JMPR's summary evaluation of studies on the short-term and long-term toxicity, reproductive and developmental toxicity, and genotoxicity and carcinogenicity of diflubenzuron. It also considered information obtained from literature searches on diflubenzuron and PCA.

The original studies provided to the 2001 JMPR were performed over a period of approximately 40 years, and all the studies were considered adequate for their intended purpose unless otherwise specified in the JMPR monograph. Some of the critical studies did not comply with GLP regulations, as the data were generated before the implementation of GLP regulations. Overall, however, the Committee considered that the database was adequate to assess the risk of diflubenzuron.

### ***Biochemical data***

Diflubenzuron is rapidly absorbed to a moderate extent from the gastrointestinal tract. In a single-dose oral study with <sup>14</sup>C-labelled diflubenzuron in rats, about 30% of the administered dose was absorbed at 5 mg/kg bw, and less was absorbed at 100 mg/kg bw. Once absorbed, diflubenzuron is extensively metabolized and rapidly excreted, mostly in the urine, although some enterohepatic circulation occurs. In the radiolabel study, more than 90% of the administered dose (5 and 100 mg/kg bw) was excreted within 24 hours. When mice were given a single oral dose of diflubenzuron at 12, 64, 200 or 920 mg/kg bw, excretion was almost complete within 48 hours.

The primary metabolic pathways are hydroxylation of the aniline ring, cleavage of the ureido bridge and conjugation, mainly with sulfate. In rats, about 80% of the metabolites were identified as involving hydroxylation of the phenyl moieties of diflubenzuron, and approximately 20% underwent scission at the ureido bridge.

PCA was not detected in bile or urine using a method with a limit of quantification (LOQ) of 7.5 ng/mL in rats. A radiolabel study with rats given a single dose of [U-<sup>14</sup>C-anilino]diflubenzuron at 104 mg/kg bw also did not identify PCA in urine. PCA was not detected (limits of detection [LODs] not given) in urine or faeces of sheep or cow following administration of a single oral dose of



diflubenzuron at 10 mg/kg bw or in rat urine following administration of a single oral dose of diflubenzuron at 5 mg/kg bw. However, when diflubenzuron was given as a single oral dose of 5 mg/kg bw, PCA was detected in small quantities in swine urine (1.03% of the oral dose) and chicken excreta (0.44% of the dose).

When diflubenzuron was given as a single oral dose, 4-chlorophenylurea (CPU), a metabolite that may be reduced to PCA, was detected in small quantities in the urine of swine (0.82% of a 5 mg/kg bw dose), in the urine of cows (0.6% of a 10 mg/kg bw dose) and in chicken excreta (3.14% of a dose of 5 mg/kg bw).

#### *Toxicological data*

Diflubenzuron was of low acute toxicity when given to mice and rats by the oral, inhalation or dermal route. The oral median lethal dose (LD<sub>50</sub>) was greater than 4600 mg/kg bw in mice and rats, the dermal LD<sub>50</sub> was greater than 10 000 mg/kg bw in rats and the inhalation median lethal concentration (LC<sub>50</sub>) was greater than 2.9 mg/L in rats.

Diflubenzuron was not irritating to the skin of rabbits and was slightly irritating to the eyes of rabbits. Diflubenzuron was not a skin sensitizer in a study in guinea-pigs.

The primary target for toxicity is the erythrocytes, with secondary effects on liver and spleen. Dose-related methaemoglobinaemia has been consistently demonstrated in both sexes of various species (mice, rats and dogs) after short-term or long-term oral exposure to diflubenzuron.

In a 13-week study, rats were fed diets containing diflubenzuron at a concentration of 0, 160, 400, 2000, 10 000 or 50 000 mg/kg feed (equivalent to 0, 8, 20, 100, 500 and 2500 mg/kg bw per day, respectively). A range of dose-related changes in erythrocyte parameters (erythrocyte counts, haemoglobin, reticulocytes, methaemoglobin and sulphaemoglobin) were noted in both sexes at 400 mg/kg feed and above, with minimal effects at 160 mg/kg feed. The absolute and relative weights of the spleen were increased in males at 160 mg/kg feed and above and in females at 400 mg/kg feed and above for 7 weeks. Pathological findings included chronic hepatitis, haemosiderosis and congestion of the spleen, and erythroid hyperplasia of the bone marrow in all treated groups; and haemosiderosis in the liver at 400 mg/kg feed and above. A NOAEL could not be identified, because there were small, but statistically significant, increases in methaemoglobin concentration and associated changes in the spleen and bone marrow at the lowest dose tested (160 mg/kg feed, equivalent to 8 mg/kg bw per day).

In a 13-week non-GLP-compliant study in dogs, animals received diets containing diflubenzuron at a concentration of 0, 10, 20, 40 or 160 mg/kg feed (equal to 0, 0.4, 0.8, 1.6 and 6.4 mg/kg bw per day, respectively). At week 6, haemoglobin

concentration and erythrocyte count were reduced and methaemoglobin and free haemoglobin concentrations were increased at 160 mg/kg feed. There was an increase in the myeloid:erythroid ratio in bone marrow at 160 mg/kg feed at week 12. A NOAEL of 40 mg/kg feed (equal to 1.6 mg/kg bw per day) was identified, based on changes in haematological end-points and bone marrow at 160 mg/kg feed (equal to 6.4 mg/kg bw per day).

In a 52-week non-GLP-compliant study, dogs received gelatine capsules containing diflubenzuron at a dose of 0, 2, 10, 50 or 250 mg/kg bw per day. A range of effects related to impaired erythrocytes was seen at the two highest doses from week 13 onwards. Increases in methaemoglobin and sulfhaemoglobin concentrations and in platelet counts were seen at 10 mg/kg bw per day and above. The only histopathological findings were in the liver (increased pigmentation of Kupffer cells and macrophages) at 10 mg/kg bw per day and above. A NOAEL of 2 mg/kg bw per day was identified, based on effects on methaemoglobin and sulfhaemoglobin concentrations, platelet counts and hepatic pigmentation at 10 mg/kg bw per day.

In a chronic toxicity and carcinogenicity study, diflubenzuron was given to mice in the diet at a concentration of 0, 16, 80, 400, 2000 or 10 000 mg/kg feed (equal to 0, 1.2, 6.4, 32, 160 and 840 mg/kg bw per day for males and 0, 1.4, 7.3, 35, 190 and 960 mg/kg bw per day for females, respectively) for 91 weeks. Significant, dose-related changes were seen in a number of haematological parameters from week 26 onwards (methaemoglobin and sulfhaemoglobin at 80 mg/kg feed and above; haemoglobin at 2000 mg/kg feed and above; leukocyte and erythrocyte counts at 10 000 mg/kg feed). On week 26, absolute spleen weights were significantly increased at 2000 mg/kg feed and above. Increased incidences of splenic siderocytes at 400 mg/kg feed and above and of pigmented Kupffer cells at 10 000 mg/kg feed were noted. A NOAEL of 16 mg/kg feed (equal to 1.2 mg/kg bw per day) was identified, based on methaemoglobin formation at 80 mg/kg feed (equal to 6.4 mg/kg bw per day). There was no evidence of carcinogenicity in this study.

In a non-GLP-compliant chronic toxicity and carcinogenicity study in rats, animals received diflubenzuron in the diet at a concentration of 0, 10, 20, 40 or 160 mg/kg feed (equivalent to 0, 0.5, 1, 2 and 8 mg/kg bw per day, respectively) for 2 years. The achieved dietary concentrations and homogeneity of diflubenzuron in the feed were not confirmed. The NOAEL was 40 mg/kg feed (equivalent to 2 mg/kg bw per day), based on increases in methaemoglobin concentration and reduced free haemoglobin concentration at 160 mg/kg feed (equivalent to 8 mg/kg bw per day). There was no increase in the incidence of tumours in treated animals. However, the poor survival (<30% in all groups at termination) and limited range of tissues examined limited the power of this study to detect any carcinogenicity of diflubenzuron.

In a GLP-compliant combined 2-year toxicity and carcinogenicity study, rats received diflubenzuron in the diet at a concentration of 0, 160, 620, 2500 or 10 000 mg/kg feed (equal to 0, 7.1, 28, 112 and 472 mg/kg bw per day for males and 0, 9.3, 37, 128 and 612 mg/kg bw per day for females, respectively). Erythrocyte parameters (e.g. methaemoglobin and sulphaemoglobin concentrations) were altered, with no marked progression with duration and dosing. The main treatment-related histopathological findings were pigmented macrophages in the spleen and liver and erythroid hyperplasia of the bone marrow at 620 mg/kg feed and above. A NOAEL for toxicity could not be identified, owing to increases in methaemoglobin and sulphaemoglobin concentrations noted at 160 mg/kg feed (equal to 7.1 mg/kg bw per day), the lowest dose tested. The overall incidences of tumours were low, with no treatment- or dose-related findings.

The overall NOAEL for toxicity in the 2-year studies in rats was 2 mg/kg bw per day, and the overall LOAEL was 7.1 mg/kg bw per day.

The Committee concluded that diflubenzuron is not carcinogenic in mice or rats.

The genotoxicity of diflubenzuron was evaluated in an adequate range of assays, both *in vitro* and *in vivo*. No evidence of genotoxicity was found, other than two recent *in vivo* studies in which positive findings in micronucleus induction and comet formation in the peripheral blood (the target of toxicity) were reported in mice given diflubenzuron at a dose of 0.3, 1 or 3 mg/kg bw. The genotoxicity potency reported in this study was inconsistent with what was reported in other studies and has not been replicated. The Committee concluded that diflubenzuron is not genotoxic based on the weight of evidence of genotoxicity information available.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Committee concluded that diflubenzuron is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study, rats received diets containing diflubenzuron at a concentration of 0, 500, 5000 or 50 000 mg/kg feed (equal to 0, 42, 430 and 4300 mg/kg bw per day for males and 0, 36, 360 and 3800 mg/kg bw per day for females, respectively). Haematological parameters and the spleen were not examined in young animals. Reproductive parameters were not affected. Pup weights were reduced in a dose-related manner in the F<sub>1</sub> generation, but not in the F<sub>2</sub> generation. Alterations in erythrocyte parameters and increases in lymphocyte counts and platelet numbers were seen in all parental groups. The spleen was the primary target organ, showing increases in weight, congestion and haemosiderosis at all doses and an increase in the incidence of congested red pulp in F<sub>0</sub> animals at the middle and high doses. Effects on liver included increased incidences of centrilobular hepatocyte hypertrophy at the middle and high doses and brown pigmentation of Kupffer cells in all treated

groups. The NOAEL for reproductive effects was 50 000 mg/kg feed (equal to 3800 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 5000 mg/kg feed (equal to 360 mg/kg bw per day), based on reductions in pup body weight at 50 000 mg/kg feed (equal to 3800 mg/kg bw per day) in the F<sub>1</sub> generation. A NOAEL for parental toxicity could not be identified because of the haematological effects observed at all doses tested.

In a developmental toxicity study, rats were dosed orally by gavage with diflubenzuron at 0 or 1000 mg/kg bw per day (the limit dose) from days 6 to 15 of gestation. The dams were killed on day 20 of gestation. No treatment-related effects on the dams or fetuses were noted. The NOAEL for both maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the only dose tested.

In another developmental toxicity study, rabbits were dosed orally by gavage with diflubenzuron at 0 or 1000 mg/kg bw per day (the limit dose) from days 7 to 19 of gestation. The does were killed on day 28 of gestation. No treatment-related effects on the does or fetuses were noted. There was no evidence of developmental toxicity in rabbits. The NOAEL for both maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the only dose tested.

#### *Observations in humans*

No reports of adverse effects or poisoning incidences associated with diflubenzuron were found.

#### *Toxicological data on PCA (a metabolite of diflubenzuron)*

Repeated exposure to PCA leads to cyanosis and methaemoglobinaemia, followed by effects in blood, liver, spleen and kidneys, as evidenced by changes in haematological parameters, splenomegaly and haemosiderosis (from moderate to heavy) in spleen, liver and kidney, partially accompanied by extramedullary haematopoiesis. The LOAELs for a significant increase in methaemoglobin levels in rats and mice were 5 and 7.5 mg/kg bw per day, respectively, for a 13-week oral gavage administration of PCA. The LOAEL for a 103-week oral gavage study in rats (with administration 5 days/week) was 2 mg/kg bw per day, based on a significant increase in methaemoglobin levels and fibrotic changes of the spleen in male rats; hyperplasia of bone marrow was observed in female rats at and above 6 mg/kg bw per day. This information demonstrated that PCA exhibits toxicity end-points similar to those of diflubenzuron, but is more potent than diflubenzuron.

PCA was tested for carcinogenicity in mice and rats by administration in the diet and by oral gavage.

In a dietary carcinogenicity study in mice, animals received PCA at a concentration of 0, 2500 or 5000 mg/kg feed (equivalent to 0, 375 and

750 mg/kg bw per day, respectively) for 78 weeks, followed by a 13-week observation period. Decreased body weight gain was observed in treated animals. Non-neoplastic proliferative and chronic inflammatory lesions were found in the spleens of treated mice. There was an increased incidence of haemangiosarcomas in the spleen, liver, kidney and subcutaneous tissue (combined) for both sexes. It was concluded that there was insufficient evidence to conclude that PCA was carcinogenic in mice.

In a second carcinogenicity study in mice, animals were administered PCA by oral gavage in aqueous hydrochloric acid at 0, 3, 10 or 30 mg/kg bw per day, 5 days/week, for 103 weeks. Incidences of proliferation of haematopoietic cells in the liver were increased in dosed females. Multifocal renal tubular pigmentation (haemosiderin) was observed in high-dose females. There were increases in the incidences of hepatocellular carcinomas in males dosed at 10 and 30 mg/kg bw per day (3/50, 7/49, 11/50, 17/50), incidences of combined hepatocellular adenomas and carcinomas in all treated males (11/50, 21/49, 20/50, 21/50) and incidences of haemangiosarcomas of the liver and spleen (combined) in males at 30 mg/kg bw per day (4/50, 4/49, 1/50, 10/50). It was concluded that there was some evidence of carcinogenicity in male mice and no evidence in female mice.

In a dietary carcinogenicity study in rats, animals received PCA at a concentration of 0, 250 or 500 mg/kg feed (equivalent to 0, 12.5 and 25 mg/kg bw per day, respectively) for 78 weeks, followed by a 24-week observation period. Mesenchymal tumours (fibroma, fibrosarcoma, haemangiosarcoma, osteosarcoma and sarcoma not otherwise specified) in the spleen were observed in males at the high dose and in females at both doses; no tumours were found in the controls. It was concluded that there was insufficient evidence to conclude that PCA was carcinogenic in rats.

In a second carcinogenicity study in rats, animals were administered PCA by oral gavage in aqueous hydrochloric acid at 0, 2, 6 or 18 mg/kg bw per day, 5 days/week, for 103 weeks. Changes in haematological parameters (e.g. decreases in haemoglobin concentration, erythrocyte count and haematocrit) were noted at various time points. Non-neoplastic findings included bone marrow hyperplasia, hepatic haemosiderosis and splenic fibrosis. The incidence of uncommon sarcomas of the spleen in high-dose male rats was significantly higher than that in the vehicle controls (fibrosarcomas, osteosarcomas or haemangiosarcomas, combined: 0/49, 1/50, 3/50, 38/50); some of these tumours metastasized to one or more sites. One mid-dose female developed fibrosarcoma, and one high-dose female developed osteosarcoma; the controls showed zero incidence of either of these tumours. The incidence of adrenal phaeochromocytomas or malignant phaeochromocytomas combined was significantly higher in the high-dose males. There was a non-significant increase in the incidence of phaeochromocytomas in

high-dose females (2/50, 3/50, 1/50, 6/50). It was concluded that there was clear evidence of carcinogenicity in male rats and equivocal evidence in female rats.

The oral gavage carcinogenicity study is considered to be more appropriate than the dietary admixture feeding study for determining carcinogenicity because (1) PCA is unstable in feed and (2) mice and rats in the feeding studies were dosed for 78 weeks and killed and examined for histopathology following a further 13-week (mice) or 24-week (rats) observation period. Nonetheless, both studies showed some similar effects: splenic toxicity in male and female rats, a treatment-related increase in uncommon splenic sarcomas in male rats and a treatment-related increase in haemangiosarcomas in male mice.

The Committee concluded that PCA is carcinogenic in mice and rats.

PCA has been tested for genotoxicity in various *in vitro* and *in vivo* systems. PCA is genotoxic *in vitro* and *in vivo*. The Committee is aware of the existence of additional *in vivo* genotoxicity studies; however, these were not available to the Committee.

The Committee concluded that PCA is genotoxic.

There is no established mode of action for PCA carcinogenesis; it is not known whether it is mediated through a genotoxic and/or non-genotoxic mechanism. Several hypotheses regarding the mechanism of splenic carcinogenicity have been proposed. However, because PCA is genotoxic and carcinogenic, the Committee could not exclude the possibility that the carcinogenesis of PCA occurs by a genotoxic mode of action.

#### *Microbiological data*

Considering the chemical structure and mode of action of diflubenzuron, the Committee did not anticipate any adverse effects of diflubenzuron residues on human gastrointestinal microbiota.

#### *Evaluation*

In the absence of adequate information on exposure to PCA, a genotoxic and carcinogenic metabolite and/or degradate of diflubenzuron, and on whether diflubenzuron can be metabolized to PCA in humans, the present Committee was unable to establish an ADI for diflubenzuron because it was not possible to assure itself that there would be an adequate margin of safety from its use as a veterinary drug. The Committee also noted that it was not possible to calculate a margin of exposure for PCA in the absence of adequate information on exposure to PCA.

*Additional information that would assist in the further evaluation of the compound*

- A comparative metabolism study of diflubenzuron in humans and rats (e.g. in hepatocytes)
- Information on PCA exposure associated with the consumption of treated fish
- Information on the amount of PCA present (if any) as an impurity in the product formulation
- Information on the amount of PCA generated during food processing.

*Recommendation*

The Committee recommended that JMPR consider the re-evaluation of diflubenzuron at a future meeting and that the WHO Pesticide Evaluation Scheme (WHOPES) and the WHO *Guidelines for Drinking-water Quality* (GDWQ) Chemical Working Group reconsider their recommendations for the use of diflubenzuron as a vector control agent in drinking-water.

A toxicological monograph was prepared.

*Residue evaluation*

The Committee reviewed studies on the pharmacokinetics and metabolism of diflubenzuron in food-producing animals, including Atlantic salmon. Also, a number of radiolabelled and non-radiolabelled diflubenzuron residue depletion studies in Atlantic salmon and Atlantic cod were reviewed. The analytical method submitted by the sponsor to support the residue monitoring has been assessed.

All studies were GLP compliant unless otherwise stated.

*Data on pharmacokinetics and metabolism*

In two studies conducted at a water temperature of 15 °C, Atlantic salmon received a single dose of [<sup>14</sup>C]diflubenzuron or multiple doses (13 days of feeding of non-radiolabelled diflubenzuron followed by a single dose of radiolabelled [<sup>14</sup>C]diflubenzuron) at a concentration of 3 mg/kg bw. Diflubenzuron was the main component of the total radioactive residue (TRR) both in fillet and in liver, corresponding to 94.8% and 72.2%, respectively, at day 1 after the repeated-dosing regimen. For the single-dose regimen, diflubenzuron represented 88.6% and 69.3% of the TRR for fillet and liver, respectively. Diflubenzuron was metabolized and rapidly excreted, mainly via the bile. Six hours after administration, 39% of the radioactivity in bile was identified as diflubenzuron. One and 4 days after administration, most of the radioactivity in bile was attributable to polar



metabolites. Chromatographic analysis with radio-high-performance liquid chromatography (HPLC) of fillet revealed three components. The major component was identified as diflubenzuron at concentrations of 389 µg/kg, 99.6 µg/kg and 21.4 µg/kg at 1, 4 and 7 days following repeat administration and 410 µg/kg at 1 day following a single administration. Furthermore, one metabolite was identified as CPU with a maximum concentration of 0.23 µg/kg at 4 days following repeat administration of diflubenzuron. The third component was not identified, but the retention time was in the same range as for PCA. Base hydrolysis of solid residues in liver revealed at least five components at concentrations lower than 9 µg/kg. Three of the components were identified as diflubenzuron, PCA (<3 µg/kg) and CPU (<9 µg/kg). The two unidentified metabolites were probably monohydroxylated products of diflubenzuron.

In a non-GLP-compliant study, Atlantic salmon smolts (weighing approximately 60 g), maintained at a water temperature of 8 °C, received a single dose of 75 mg/kg bw of [<sup>14</sup>C]diflubenzuron via gavage. After 2 hours, 12 hours, 2 days, 6 days, 10 days, 13 days, 20 days and 27 days, fish were slaughtered, and 1–2 fish were sampled for autoradiography. Samples were taken from blood, brain, muscle, abdominal fat, kidney, liver, bile, cartilage and cutaneous mucus. The concentration of radioactivity in brain and cartilage was highest 12 hours after administration, with concentrations of 13.8 µg/g and 10.9 µg/g, respectively. In bile, the concentration of radioactivity varied between 275 and 1066 µg/g in the first 10 days after administration, then dropped to less than 4 µg/g for the rest of the period.

#### *Residue data*

The Committee reviewed residue depletion studies for Atlantic salmon and Atlantic cod.

**Salmon.** Two residue depletion studies using radiolabelled diflubenzuron and three residue depletion studies using non-radiolabelled diflubenzuron were provided for Atlantic salmon, using a single oral dose or repeated dose. Whereas the residue depletion studies with radiolabelled diflubenzuron were conducted at a water temperature of 15 °C, the studies with the non-radiolabelled drug were carried out at two water temperatures: 15 °C and 6 °C.

In the first study, Atlantic salmon (440–851 g) held at a water temperature of 15 °C received a dose of 3 mg/kg bw of [<sup>14</sup>C]diflubenzuron by gavage. Liver, fillet (muscle and skin), gallbladder (including bile) and residual carcass were collected from 10 fish each at 1 and 7 days post final dose administration. Samples of tissues were collected for TRR determination using liquid scintillation counting. The LODs were 2 µg equivalent (eq)/kg for liver and 0.6 µg eq/kg for



fillet and carcass. Acetonitrile and ethyl acetate tissue sample extracts were also analysed using reversed-phase HPLC with an ultraviolet (UV) detector operating at 254 nm. Fish fillet extracts were analysed by liquid chromatography with mass spectrometry (LC-MS). Diflubenzuron was found as the main TRR both in fillet and in liver, corresponding to 88.6% and 69.3% of the TRR for fillet and liver, respectively.

In a second study, Atlantic salmon weighing 514–863 g received a non-radiolabelled diflubenzuron dose of 3 mg/kg bw per day for 13 consecutive days followed by a single dose of 3 mg/kg bw radiolabelled [<sup>14</sup>C]diflubenzuron by gavage. Liver, fillet (muscle and skin), gallbladder (including bile) and residual carcass were collected from 10 fish each at 1, 4 and 7 days post final dose administration. The analyses were carried out as described in the first study. Diflubenzuron was found as the main TRR component both in fillet and in liver, corresponding to 94.8% and 72.2%, respectively, at day 1 post-treatment. The highest concentration of radioactivity (mean ± standard deviation [SD]) in liver (811 ± 100 µg eq/kg), fillet (466 ± 66 µg eq/kg) and carcass (734 ± 118 µg eq/kg) occurred 1 day after administration of the drug.

In both studies with radiolabelled diflubenzuron, the highest concentrations of [<sup>14</sup>C]diflubenzuron equivalents occurred at 1 day post-treatment. Less than 20% of the radiochemical dose remained in the liver, fillet and carcass 1 day following repeated administration, and less than 33% remained following a single dose administration. The concentrations decreased to less than 1.5% by 7 days following both dosing regimens. The major metabolic pathway is excretion of the parent compound.

At a water temperature of 15 °C, the MR:TRR ratio at day 1 post-dose was 0.91 in muscle in the single-dose regimen. For the repeated dose, the MR:TRR ratios were 0.96, 0.88 and 0.94 for 1, 4 and 7 days post last dose.

In a study using non-radiolabelled diflubenzuron in feed, Atlantic salmon (600–987 g), maintained at a water temperature of 6 °C, received a dose of 3 mg/kg bw for 14 consecutive days. Tissues (liver, muscle and skin) were collected and analysed after 1, 7, 14 and 21 days post-treatment. Diflubenzuron was extracted from the tissues by solid-liquid extraction with acetonitrile. Samples were analysed using a validated HPLC-UV method. The average concentrations of diflubenzuron in fillet were 2240 µg/kg, 400 µg/kg, 100 µg/kg and <LOQ (50 µg/kg) on days 1, 7, 14 and 21 post-treatment, respectively. The average concentrations of diflubenzuron in liver were 3190 µg/kg, 730 µg/kg, 120 µg/kg and <LOQ on days 1, 7, 14 and 21 post-treatment, respectively.

The same protocol as described previously was used for another study at 15 °C, where Atlantic salmon (600–987 g) received non-radiolabelled diflubenzuron in feed daily at a dose of 3 mg/kg bw for 14 consecutive days. Diflubenzuron was quantified in muscle and liver by the same HPLC-UV method

described in the previous study. The average concentrations of diflubenzuron determined in fillet were 1550 µg/kg and 200 µg/kg on days 1 and 7, respectively, and <LOQ on days 14 and 21 post-treatment. The average concentrations of diflubenzuron in liver were 2170 µg/kg and 260 µg/kg on days 1 and 7 post-treatment, respectively, and <50 µg/kg (LOQ) after 14 days post-treatment.

In another depletion study carried out at 15 °C, Atlantic salmon (weighing 4.6–5.6 kg) received non-radiolabelled diflubenzuron at 3 mg/kg bw for 14 consecutive days. Liver, muscle and skin samples were collected during the treatment with the medicated feed (days 3, 7 and 14) and days 5, 14, 21 and 28 post-treatment and then analysed. Results for samples taken post-treatment are as follows: In liver, the average diflubenzuron concentrations (10 fish) were 520 µg/kg and 70 µg/kg on days 5 and 14, respectively. In muscle, the average concentrations were 900 µg/kg and 100 µg/kg on days 5 and 14, respectively. In skin, the average concentrations were 320 µg/kg and <50 µg/kg on days 5 and 14, respectively. At 21 days post-treatment, all samples analysed had diflubenzuron concentrations lower than 50 µg/kg (LOQ). In samples taken during the treatment, the highest average diflubenzuron concentration was determined at day 14 in liver (1820 µg/kg) and muscle (2130 µg/kg). For skin, the highest diflubenzuron concentration of 1320 µg/kg was reached on day 7. The maximum diflubenzuron concentration of 3700 µg/kg was found in one muscle sample on day 14.

**Atlantic cod.** In a non-GLP-compliant residue depletion study, Atlantic cod (*Gadus morhua*) (81–122 g), maintained at a water temperature of  $7.7 \pm 0.2$  °C, received a dose of 3 mg/kg bw of diflubenzuron in feed for 14 consecutive days. The highest concentration of diflubenzuron in liver ( $181 \pm 21$  ng/g) was observed 1 day after the end of the treatment (day 15).

In another non-GLP-compliant study conducted at a water temperature of 7.7 °C, Atlantic cod (65–165 g) received diflubenzuron in feed at a dose of 3 mg/kg bw for 14 consecutive days. Samples of fillet and skin in natural proportion, liver and terminal colon were taken on days 4, 8 and 12 during the treatment and days 1, 4, 8, 15, 22 and 30 post-treatment. At each time point, 10 fish were collected and analysed individually, with the exception of the bile samples, which were combined into one or two group samples for each sampling day. Diflubenzuron was quantified by LC-MS using teflubenzuron as the internal standard. The LOQ of the validated method was 20 µg/kg. The calculated tissue concentrations in the samples showed high variability, probably due to individual differences in feed consumption and, to a lesser extent, in absorption. The median concentration determined in fillet and skin throughout the treatment period was 36.1 µg/kg, only 1.5% of the mean concentration determined in Atlantic salmon fillet after the same treatment, which suggests that diflubenzuron has a lower gastrointestinal uptake in Atlantic cod compared with Atlantic salmon. The depletion half-lives

for diflubenzuron in fillet and liver ranged from 0.8 to 0.9 day. PCA was not detected (LOD 2 µg/kg) in any samples analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

#### *Analytical methods*

The Committee assessed the validation data against the requirements for analytical methods as published in CAC/GL 71-2009 (15).

The validated HPLC-UV method used in the depletion studies is based on solid-liquid extraction, followed by several clean-up steps using liquid-liquid extraction and solid-phase extraction on a Florisil sorbent. Quantification was performed at 254 nm using an external calibration curve. Average recoveries of 88% for liver, 91% for muscle (values corrected for blank) and 103% for skin were determined. The LOD and LOQ were 20 µg/kg and 50 µg/kg, respectively. The Committee concluded that the HPLC-UV method provided by the sponsor lacks in selectivity because of possible interferences from other components in the extract at a wavelength of 254 nm and cannot be recommended for regulatory monitoring of salmon tissues for diflubenzuron.

Several multi-residue analytical methods are reported in the peer-reviewed scientific literature. These methods are based on solvent extraction (acetone, acetonitrile or methanol) with or without hexane liquid-liquid extraction to remove fat, followed by clean-up over C18 or silica gel solid-phase extraction cartridges and determination by LC-MS/MS or using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach for the sample preparation. However, the Committee noted that the methods reported in the peer-reviewed scientific literature did not meet the analytical method validation requirements as published in CAC/GL 71-2009 (15).

#### *Maximum residue limits*

The Committee noted that PCA is a potential hydrolysis product of 4-chlorophenyl isocyanate, which is one of the starting materials for the synthesis of diflubenzuron, and that PCA could be formed through degradation of diflubenzuron at high temperatures during processing of feed or food. The data available to the Committee at the time of the assessment were inadequate regarding the formation or presence of PCA in fish, as well as in processed food.

MRLs for diflubenzuron could not be recommended by the Committee, as the Committee was unable to establish an ADI for diflubenzuron.

The Committee also noted that there is no analytical method suitable for regulatory monitoring purposes.

### Additional information that would assist in the further evaluation of the compound

- A method suitable for monitoring diflubenzuron residues in fish muscle and fillet (muscle plus skin in natural proportion).

A residue monograph was prepared.

## Summary and conclusions

### Studies relevant to risk assessment

Species / study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
<b>Diflubenzuron</b>				
<b>Mouse</b>				
Ninety-one-week toxicity and carcinogenicity study (diet)	Males: 0, 1.2, 6.4, 32, 160, 840	Toxicity: Increased methaemoglobin concentration	1.2	6.4
	Females: 0, 1.4, 7.3, 35, 190, 960	Carcinogenicity: None	840 <sup>a</sup>	–
<b>Rat</b>				
Two-year toxicity and carcinogenicity studies <sup>b</sup> (diet)	Study 1: 0, 0.5, 1, 2, 8	Toxicity: Increased methaemoglobin and sulphaemoglobin concentrations	2 <sup>c</sup>	7.1 <sup>d</sup>
	Study 2: Males: 0, 7.1, 28, 112, 472 Females: 0, 9.3, 37, 128, 612	Carcinogenicity: None	472 <sup>a</sup>	–
Two-generation reproductive toxicity study (diet)	Males: 0, 42, 430, 4 300	Reproductive toxicity: None	3 800 <sup>a</sup>	–
	Females: 0, 36, 360, 3 800	Parental toxicity: Changes in haematological parameters Offspring toxicity: Decreased F <sub>1</sub> pup weights	– 360	36 <sup>e</sup> 3 800
Developmental toxicity study (gavage)	0, 1 000	Maternal: None	1 000 <sup>f</sup>	–
		Embryo/fetal toxicity: None	1 000 <sup>f</sup>	–
<b>Rabbit</b>				
Developmental toxicity study (gavage)	0, 1 000	Maternal toxicity: None	1 000 <sup>f</sup>	–
		Embryo/fetal toxicity: None	1 000 <sup>f</sup>	–
<b>Dog</b>				
One-year toxicity study (capsule)	0, 2, 10, 50, 250	Increased methaemoglobin and sulphaemoglobin concentrations, platelet counts and hepatic pigmentation	2	10
<b>4-Chloroaniline (PCA)</b>				
<b>Mouse</b>				
Two-year carcinogenicity study (gavage)	0, 3, 10, 30	Increased incidence of hepatocellular adenomas and carcinomas in male mice	–	3 <sup>c</sup>

Species / study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Rat				
Two-year carcinogenicity study (gavage)	0, 2, 6, 18	Increased incidence of spleen sarcoma in male rats	6	18

<sup>a</sup> Highest dose tested.

<sup>b</sup> Two or more studies combined.

<sup>c</sup> Overall NOAEL.

<sup>d</sup> Overall LOAEL.

<sup>e</sup> Lowest dose tested.

<sup>f</sup> Limit dose.

## ADI

In the absence of adequate information on exposure to PCA, a genotoxic and carcinogenic metabolite and/or degradate of diflubenzuron, and on whether diflubenzuron can be metabolized to PCA in humans, the present Committee was unable to establish an ADI for diflubenzuron because it was not possible to assure itself that there would be an adequate margin of safety from its use as a veterinary drug.

## MRLs

MRLs for diflubenzuron could not be recommended by the Committee, as no ADI was established and there is a lack of data about the metabolite/degradation product PCA.

## 4.2 Ivermectin

### Explanation

Ivermectin (CAS no. 70288-86-7) is a macrocyclic lactone that is a member of the avermectin series and is widely used as a broad-spectrum antiparasitic endectocide against nematode and arthropod parasites in food-producing animals. In human medicine, ivermectin is used to treat onchocerciasis, lymphatic filariasis, strongiloidiasis and scabies. Ivermectin consists of two homologous compounds, 22,23-dihydroavermectin B<sub>1a</sub> (H<sub>2</sub>B<sub>1a</sub> or ivermectin B<sub>1a</sub>) and 22,23-dihydroavermectin B<sub>1b</sub> (H<sub>2</sub>B<sub>1b</sub> or ivermectin B<sub>1b</sub>), in the H<sub>2</sub>B<sub>1a</sub>:H<sub>2</sub>B<sub>1b</sub> ratio of 80:20. Ivermectin is used in cattle, sheep, goats, pigs, horses, reindeer and American bison at doses of 0.1–0.5 mg/kg bw given subcutaneously, topically or orally as a single-dose treatment only. Withdrawal periods range from 14 to 122 days where ivermectin is approved for use.

Ivermectin was previously considered by the Committee at its thirty-sixth, fortieth, fifty-eighth, seventy-fifth and seventy-eighth meetings ([Annex 1](#), references 91, 104, 157, 208 and 217). At its fortieth meeting, the Committee

established an ADI of 0–1 µg/kg bw based on developmental toxicity of ivermectin in CF-1 mice and recommended MRLs of 40 µg/kg for fat, 100 µg/kg for liver and 10 µg/kg for milk as ivermectin in cattle (using the marker ivermectin B<sub>1a</sub>). At its seventy-eighth meeting, the Committee recommended an MRL of 4 µg/kg for cattle muscle based on 2 × LOQ of the analytical method.

At its seventy-fifth meeting, the Committee concluded that there was a need to evaluate the toxicological information on ivermectin with a view to identifying a critical effect other than in the CF-1 mouse for the establishment of an ADI. CCRVDF at its Twenty-second Session requested that JECFA re-evaluate the ADI and the MRLs in all cattle tissues (2). CCRVDF noted that the draft MRL for ivermectin in bovine muscle recommended at the seventy-eighth meeting was in some cases ≥2.5 times lower than the MRL established in other countries where ivermectin was used. This did not reflect GVP. Furthermore, JECFA had not recommended an MRL for bovine kidney.

### ***Toxicological and microbiological evaluation***

The Committee considered data from a safety, tolerability and pharmacokinetics study in humans and information on various repeated-dose ivermectin treatment regimens in patients, which were provided by a sponsor. The Committee also considered previous evaluations by JECFA on ivermectin in various animal species and the pharmacokinetics of ivermectin in dogs in particular, so that a more appropriate animal model could be used to establish an ADI. In light of the possibility for acute exposure to high concentrations of ivermectin from the injection site, the Committee also considered the acute toxicity of ivermectin with a view to establishing an ARfD.

The critical animal studies were not performed to GLP because the data were generated prior to the implementation of GLP. The human study was conducted according to the principles of the Declaration of Helsinki. The Committee considered that the database was adequate for the evaluation.

### ***Biochemical data***

Pharmacokinetics data were obtained from the submitted safety, tolerability and pharmacokinetics study in humans (16). Twelve fasted subjects per dosing group were given a single oral dose of 30, 60, 90 or 120 mg ivermectin or multiple doses of either 30 or 60 mg ivermectin over 7 days. Pharmacokinetic analysis revealed that ivermectin concentrations in plasma were generally proportional up to a single oral dose of 120 mg in fasted subjects. Dose linearity of the maximum concentration in plasma ( $C_{\max}$ ) and the area under the plasma concentration–time curve (AUC) was confirmed after dose normalization. There were no differences in pharmacokinetic variables between men and women. Minimal accumulation

was observed with repeated dosing, and the elimination half-life ranged from 18.8 to 20.1 hours in fasted subjects. The ivermectin AUC was 2.57-fold greater in fed versus fasted subjects receiving a single dose of 30 mg, but the elimination half-life was shorter, at 15.0 hours.

A literature search identified a number of oral dosing studies in dogs that had relevant pharmacokinetics data from a test group treated with ivermectin. The data indicate that for a 3-fold increase in ivermectin dose (81.5–250 µg/kg bw per day), there was approximately a 5.5-fold increase in the ivermectin  $C_{\max}$  (24–132.6 ng/mL) and a 6-fold increase in the plasma ivermectin AUC (38.9–237 ng·d/mL). Dose normalization of the pharmacokinetics data from the oral dosing studies in dogs confirmed non-linear plasma pharmacokinetics of ivermectin in dogs. Furthermore, for this dose range, the elimination half-life ranged from 3.3 to 4.4 days.

### ***Toxicological data***

Repeated-dose studies with ivermectin in laboratory animals have previously been evaluated by JECFA ([Annex 1](#), reference 91). The findings from the most relevant of these non-GLP-compliant studies are summarized below.

In a 14-week study, ivermectin was given orally to rat pups 3–4 weeks of age, obtained from dams treated with ivermectin, at a dose of 0, 0.4, 0.8 or 1.6 mg/kg bw per day. Spleen enlargement and reactive bone marrow hyperplasia were observed in one animal at 0.8 mg/kg bw per day and in three animals at 1.6 mg/kg bw per day. Based on these observations, a LOAEL of 0.8 mg/kg bw per day was identified, and the NOAEL was 0.4 mg/kg bw per day. The Committee noted that the study design was not clearly explained and the findings were difficult to interpret.

In a 14-week study, ivermectin was given to dogs (four of each sex per group) by oral gastric intubation at a dose of 0, 0.5, 1.0 or 2.0 mg/kg bw per day. Controls received water or vehicle (sesame oil). At 2.0 mg/kg bw per day, mydriasis was observed in all animals; three males and one female developed tremors, ataxia, anorexia and dehydration, lost body weight (1.0–1.6 kg), were frequently found laterally recumbent and were ataxic when standing. Based on occasional mydriases and a retardation of weight gain in animals, the LOAEL was 1.0 mg/kg bw per day, and the NOAEL was 0.5 mg/kg bw per day (17).

In a 16-day oral toxicity study, ivermectin was given to immature rhesus monkeys (13–21 months old) at a dose of 0, 0.3, 0.6 or 1.2 mg/kg bw by nasogastric intubation. Controls received vehicle (sesame oil). There were no treatment-related effects noted in any of the treated animals. A NOAEL of 1.2 mg/kg bw per day, the highest dose tested, was identified.

Long-term oral toxicity or carcinogenicity studies with ivermectin were not available, but the Committee at its thirty-sixth meeting ([Annex 1](#), reference 91) concluded that given the structural similarities and comparative toxicological profiles of ivermectin and abamectin, such studies were not required. In a 94-week dietary carcinogenicity study in mice using abamectin doses of 0, 2, 4 and 8 mg/kg bw per day, a no-observed-effect level (NOEL) (NOAEL) of 4.0 mg/kg bw per day was identified. Furthermore, in a 105-week dietary carcinogenicity study in rats with abamectin doses of 0, 0.75, 1.5 and 2.0 mg/kg bw per day, a NOEL (NOAEL) of 1.5 mg/kg bw per day was identified. The present Committee agrees with these conclusions.

In a reproductive toxicity study, ivermectin was given orally to female rats at a dose of 0, 0.4, 0.8 or 1.6 mg/kg bw per day from 15 days prior to mating until 20 days postpartum. Two control groups received the vehicle (sesame oil). Based on no adverse findings in the dams, a maternal toxicity NOAEL of 1.6 mg/kg bw per day, the highest dose tested, was identified. A statistically significant, treatment-related increase in pup mortality in the 1.6 mg/kg bw per day group was observed on day 1 and days 7–14 postpartum. An offspring toxicity LOAEL of 1.6 mg/kg bw per day was identified, and the offspring toxicity NOAEL was 0.8 mg/kg bw per day.

Three multigeneration reproductive toxicity studies were undertaken in rats. The first two studies failed to establish a NOAEL for ivermectin when given orally to rats at 0.4, 1.2 and 3.6 mg/kg bw per day or 2.0 mg/kg bw per day, respectively. In a third multigeneration study, rats were given ivermectin orally at 0.05, 0.1, 0.2 or 0.4 mg/kg bw per day. A vehicle control group received sesame oil. Treatment-related effects were limited to a slight, but statistically significant, decrease in body weight gain during the post-weaning period in the  $F_{1b}$  females in the 0.4 mg/kg bw per day group and the  $F_{2b}$  males in the 0.2 and 0.4 mg/kg bw per day groups. There were no treatment-related effects on the reproductive performance of male or female rats in any dose group. There was no evidence of teratogenicity in the  $F_3$  offspring. A parental and offspring toxicity NOAEL of 0.4 mg/kg bw per day, the highest dose tested, was identified.

#### *Observations in humans*

In a double-blind, randomized, placebo-controlled, multiple-rising-dose study (0, 30, 60, 90 and 120 mg, equivalent to 0, 0.4, 0.8, 1.2 and 1.5 mg/kg bw, respectively, based on median body weight) to investigate the safety, tolerability and pharmacokinetics of multiple doses of ivermectin, 12 healthy human subjects per dose group were administered oral doses of ivermectin of 30 or 60 mg on days 1, 4 and 7 or single doses of 90 or 120 mg. An additional four healthy human subjects per dose group were administered a placebo. All subjects were



fasted prior to dosing. A group of the subjects who received 30 mg were allowed a 1-week washout and then fed prior to administration of a single oral dose of 30 mg ivermectin. All doses of ivermectin were well tolerated. No adverse effects on human health, in particular upon the neurological system, were identified. The NOAEL for acute oral toxicity of ivermectin was determined to be 120 mg, equivalent to 1.5 mg/kg bw, the highest dose tested, based on a median body weight of 77.9 kg (16).

Ivermectin has been administered to several million human patients for the treatment of onchocerciasis at a recommended oral dose level of 150 µg/kg bw administered once every 12 months. No signs of acute central nervous system toxicity have been reported. The adverse reactions that have been observed in treated patients have been described as allergic or inflammatory responses resulting from the killing of microfilariae, referred to as the “Mazotti reaction”. No significant adverse effects on fetuses have been reported when pregnant women were inadvertently treated with ivermectin.

Ivermectin may also be used in the treatment of lymphatic filariasis, strongiloidiasis and scabies. The treatment of scabies generally requires a single oral dose of 200 µg/kg bw, but two or three repeated doses may be needed, separated by an interval of 1 or 2 weeks, to be fully effective. The sponsor identified a number of reported studies where parasitized patients received up to 13 oral doses of ivermectin (800 µg/kg bw) during the course of treatment. These studies reported that ivermectin was well tolerated and noted no serious adverse health effects. A recent review of the acute toxicity of macrocyclic lactones reported that adverse health effects of ivermectin treatment in patients with onchocerciasis were related not to the dosage of ivermectin, but to the skin microfilarial load.

#### *Microbiological data*

Considering the chemical structure and mode of action, the Committee did not anticipate any adverse effects of ivermectin residues on human gastrointestinal microbiota.

#### *Evaluation*

**Acceptable daily intake.** The Committee established an ADI of 0–10 µg/kg bw on the basis of a NOAEL of 0.5 mg/kg bw per day for neurological effects (mydriasis) and retardation of weight gain in a 14-week dog study (17), with application of an uncertainty factor of 50. The previous ADI of 0–1 µg/kg bw is withdrawn.

The Committee did not consider the human clinical data sufficient to assess the possible long-term effects of repeated exposure to ivermectin, such as would occur from its use as a veterinary drug. Therefore, the Committee

identified the 14-week dog study as the most appropriate for use in establishing an ADI, given the non-relevance of effects in the CF-1 mouse and the neonatal rat due to their low expression of P-glycoprotein.

As the interspecies differences in pharmacokinetics between dogs and humans are such that humans would be exposed to less ivermectin at a given dose compared with dogs, a reduction in the interspecies uncertainty factor for pharmacokinetics would be appropriate. The quality of the information on pharmacokinetics in dogs was not sufficient to enable the Committee to calculate accurately a chemical-specific adjustment factor for interspecies differences in pharmacokinetics. A reduction by 50% was used as a conservative estimate. An uncertainty factor of 50, comprising a factor of 5 for interspecies differences and a factor of 10 for intraspecies differences, was therefore adopted.

**Acute reference dose.** As ivermectin may be administered to cattle in an injectable form, there is the possibility that humans may be exposed to animal tissue containing high concentrations of ivermectin from the injection site. For this scenario, the Committee evaluated the acute toxicity of the compound to determine the need for establishing an ARfD.

The Committee established an ARfD of 200 µg/kg bw, based on a NOAEL of 1.5 mg/kg bw, the highest dose tested in a safety, tolerability and pharmacokinetics study in healthy human subjects (16), with application of an uncertainty factor of 10 for intraspecies variability. The Committee identified the human study as the most appropriate study for use in establishing an ARfD, given the non-relevance of the embryo/fetal toxicity findings in juvenile rats due to their low expression of P-glycoprotein. The Committee noted that the ARfD was conservative, as an acute oral LOAEL for ivermectin has not been identified in humans.

An addendum to the toxicological monograph was prepared.

### **Residue evaluation**

The current Committee received seven residue depletion studies from two sources. Six had not been previously reviewed by JECFA, and one was resubmitted from the original sponsor.

All studies in this report are GLP compliant unless otherwise indicated.

### **Resubmitted study**

In a study using non-radiolabelled drug, 36 castrated male and 36 female crossbred beef cattle weighing 297–401 kg were used. A 1% weight per volume (w/v) ivermectin injectable formulation was administered subcutaneously at

1 mL/50 kg. Animals were killed in groups of 12 at 21, 28, 35, 42, 49 and 56 days post-dose, and edible tissues, including injection site, were collected from each animal. The samples were analysed by a validated HPLC method with fluorescence detection. The LOD and “limit of reliable measurement”, assumed to be the LOQ, were 1–2 µg/kg and 10 µg/kg, respectively. Residues were highest in liver, followed by fat. Residues had depleted to below the LOQ of the method in liver by 49 days post-dose. In muscle and kidney, residue concentrations had depleted to below the LOQ by 21 days post-dose.

*New studies submitted for consideration by current JECFA*

A non-GLP-compliant radiolabelled residue depletion study was conducted using [<sup>3</sup>H]ivermectin pour-on formulation. Twelve steers were dosed at 0.5 mg/kg bw with a topical formulation of 0.5% w/v ivermectin at a specific activity of 11.1 MBq/mg and a 93:7 ratio of H<sub>2</sub>B<sub>1a</sub>:H<sub>2</sub>B<sub>1b</sub>. Three animals in each group were slaughtered at 7, 14, 28 or 42 days post-dose to collect edible tissues and excreta. The TRR concentrations were determined by combustion analysis. The drug was excreted mainly in faeces, with a much lower percentage excreted in urine. The residue concentrations were highest in liver, followed by fat, muscle adjacent to the dose site, kidney and, lastly, regular muscle.

In a non-radiolabelled residue depletion study, 40 cattle (20 females, 20 males) weighing 255–382 kg were administered a single subcutaneous injection of a combination product at a dose of 0.2 mg ivermectin/kg bw plus 2 mg clorsulon/kg bw. Tissue samples (entire liver, both kidneys, perirenal fat, skeletal muscle, core injection site and concentric ring around the core injection site) were collected on days 3, 10, 17, 28, 45, 52, 60, 70, 79 and 80 post-dose. Tissue samples were assayed for determination of ivermectin marker residue (ivermectin B<sub>1a</sub>) using a validated analytical method utilizing HPLC with fluorescence detection. The validated LOQ for the marker residue was 5 µg/kg, and the LOD was 1 µg/kg. The injection site core muscle had the highest residues among all analysed tissues, followed by the liver, fat, kidney and regular muscle, consistent with the distribution pattern observed in the previously described studies. At 60 days post-dose, residues were still found in some liver and fat samples. Although sampling continued through 80 days post-dose, no samples were analysed beyond 60 days post-dose.

An additional three studies of unknown GLP status and one GLP-compliant study conducted in cattle were also submitted. Although lacking sufficient information to be considered suitable in the development of MRL recommendations, these studies provided supporting information consistent with more well-documented studies.

On the basis of the deficiencies identified by the Committee in four of the new depletion studies submitted for consideration by the eighty-first JECFA, only one new depletion study with non-radiolabelled drug together with the new radiolabel study and the depletion study with non-radiolabelled drug previously submitted to JECFA were used in the development of MRL recommendations.

The Committee confirmed that ivermectin B<sub>1a</sub> is the marker residue and that liver and fat are the target tissues for the use of ivermectin in cattle.

### *Analytical methods*

A reversed-phase HPLC method with fluorescence detection was used to determine the marker residue (ivermectin B<sub>1a</sub>) in bovine edible tissues. After tissue homogenization in acetone/water, the marker residue is extracted with isooctane. After evaporation, fat is removed from the sample with acetonitrile/hexane binary mixture. The solvent is removed by evaporation, and a fluorescent derivative is formed by on-line derivatization with trifluoroacetic anhydride/*N*-methylimidazole. The derivatized residue is assayed using HPLC/fluorescence with an excitation wavelength of 365 nm and an emission wavelength of 470 nm. No internal standard is used. The method quantitatively measures the B<sub>1a</sub> component of ivermectin by comparison with a series of derivatized ivermectin external standards.

The Committee assessed the validation data against the analytical requirements as published in CAC/GL 71-2009 (15). The method has been validated for selectivity, precision and accuracy, LOD and LOQ. No interfering peaks were observed at the retention time of ivermectin B<sub>1a</sub> in any of the non-fortified tissue samples, attesting to the selectivity of the method. The response of the method was linear over the range 5–1000 µg/kg. Calculated LODs were 0.10 µg/kg for fat, 0.10 µg/kg for kidney, 0.10 µg/kg for liver and 0.05 µg/kg for muscle. The LOD of the method was set at 1 µg/kg (lowest analysed concentration). The LOQ was set at 5 µg/kg for all tissues, the lowest concentration validated for ivermectin B<sub>1a</sub> with an acceptable precision and accuracy.

The Committee considered the quantitative HPLC/fluorescence method submitted by the sponsor to be suitably validated to support the MRLs recommended by the present meeting of the Committee. In addition, the Committee noted the availability of LC-MS/MS methods, such as the method reviewed by the seventy-eighth meeting of the Committee ([Annex 1](#), reference 217), which meet both quantitative and confirmatory requirements for the determination of ivermectin B<sub>1a</sub> residues in bovine muscle.

*Maximum residue limits*

In recommending MRLs for ivermectin in cattle, the Committee considered the following factors:

- The ADI established by the Committee was 0–10 µg/kg bw.
- An ARfD of 200 µg/kg bw was established by the Committee.
- Ivermectin B<sub>1a</sub> (synonym for 22,23-dihydroavermectin B<sub>1a</sub>) is confirmed as the marker residue.
- The ratios of the marker residue to total residues of 0.18 in fat, 0.54 in kidney, 0.37 in liver and 0.67 in muscle defined by the fortieth JECFA were confirmed.
- Two studies were used for deriving the MRLs and represent different formulations and routes of administration of ivermectin to cattle.
- The analysis of all data in cattle shows comparable residue depletion profiles.
- A validated quantitative analytical method for all edible tissues is available and is suitable for regulatory monitoring.
- The MRLs recommended for cattle tissues are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile of residue concentrations (95/95 upper tolerance limit, or UTL) for the day 14 post-treatment data from the non-radiolabelled residue depletion studies. The time point chosen is consistent with approved uses (GVP).

Based on the new assessment, the Committee recommended the following revised MRLs in cattle tissues: 400 µg/kg for fat, 100 µg/kg for kidney, 800 µg/kg for liver and 30 µg/kg for muscle.<sup>3</sup>

*Chronic dietary exposure assessment*

The EDI is 38 µg/person per day, based on a 60 kg individual, which represents 6% of the upper bound of the ADI.

The GECDE for the general population is 0.9 µg/kg bw per day, which represents 9% of the upper bound of the ADI. The GECDE for children is 1.5 µg/kg bw per day, which represents 15% of the upper bound of the ADI. The GECDE for infants is 1.3 µg/kg bw per day, which represents 13% of the upper bound of the ADI.

<sup>3</sup> No new data were provided for use of ivermectin in dairy cattle; therefore, the Committee did not recommend any revision to the MRL of 10 µg/kg for ivermectin in milk.

### *Acute dietary exposure assessment: injection site residues*

A combined analysis of all studies submitted showed that after 14 days, the maximum values of residues found at injection sites led to acute exposures (GEADE) of 52 µg/kg bw for the general population and 87 µg/kg bw for children, corresponding, respectively, to 27% and 43% of the ARfD.

The Committee considers that the presence of high concentrations of ivermectin residues at the injection site is product dependent and must be assessed on a case-by-case basis during marketing authorization by comparison of suitable acute dietary exposure estimates with the ARfD.

A residue monograph was prepared.

### **Summary and conclusions**

#### *Studies relevant to risk assessment*

Species / study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
<b>Rat</b>				
Reproductive toxicity study (oral)	0, 0.4, 0.8, 1.6	Increase in pup mortality	0.8	1.6
Multigeneration reproductive toxicity study (oral)	0, 0.05, 0.1, 0.2, 0.4	No adverse findings	0.4 <sup>a</sup>	–
<b>Dog</b>				
Fourteen-week toxicity study (gastric intubation)	0, 0.5, 1.0, 2.0	<b>Mydriasis, retarded body weight gain</b>	<b>0.5*</b>	1.0
<b>Rhesus monkey</b>				
Sixteen-day toxicity study (nasogastric intubation)	0, 0.3, 0.6, 1.2	No adverse findings	1.2 <sup>a</sup>	–
<b>Human</b>				
Safety, tolerability and pharmacokinetics study in healthy subjects (oral)	0, 0.4, 0.8, 1.2, 1.5	<b>No adverse findings</b>	<b>1.5<sup>a,**</sup></b>	–

\* Pivotal study value for the derivation of the ADI (17)

\*\* Pivotal study value for the derivation of the ARfD (16)

<sup>a</sup> Highest dose tested.

#### *Uncertainty factor*

ADI: 50 (5 for interspecies variability and 10 for intraspecies variability)

ARfD: 10 (10 for intraspecies variability)

*ADI (based on toxicological effects)*

0–10 µg/kg bw

*ARfD (based on toxicological effects)*

200 µg/kg bw

*Residue definition*

Ivermectin B<sub>1a</sub> (22,23-dihydroavermectin B<sub>1a</sub>)

*MRLs*

400 µg/kg for fat, 100 µg/kg for kidney, 800 µg/kg for liver and 30 µg/kg for muscle in cattle

*Estimated chronic dietary exposure*

The EDI is 38 µg/person per day, based on a 60 kg individual, which represents 6% of the upper bound of the ADI. The GECDE for the general population is 0.9 µg/kg bw per day, which represents 9% of the upper bound of the ADI. The GECDE for children is 1.5 µg/kg bw per day, which represents 15% of the upper bound of the ADI. The GECDE for infants is 1.3 µg/kg bw per day, which represents 13% of the upper bound of the ADI.

*Estimated acute dietary exposure*

After 14 days, the maximum values of residues found at injection sites led to acute exposures (GEADE) of 52 µg/kg bw for the general population and 87 µg/kg bw for children, corresponding, respectively, to 27% and 43% of the ARfD.

## 4.3 Sisapronil

### *Explanation*

Sisapronil, formerly known as phenylpyrazole, is the proposed International Non-proprietary Name (INN) for 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[2,2-difluoro-1-(trifluoromethyl) cyclopropyl]-1H-pyrazole-3-carbonitrile (IUPAC), with CAS no. 856225-89-3. It is a new member of the phenylpyrazole class of compounds. It is a long-acting subcutaneous injectable ectoparasiticide for control of cattle ticks. It also aids in the control of bot fly larvae, hornfly and screwworm. It is approved for use in cattle as a single subcutaneous injection of 2 mg/kg bw, with a withdrawal time of 120 days.

Sisapronil binds to ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA), thereby non-competitively blocking pre-synaptic and post-synaptic transfer of chloride ions across cell membranes in insects or acari. This mechanism of action results in uncontrolled activity of the central nervous system and death of the parasites.

Sisapronil has not previously been evaluated by the Committee. The Committee evaluated sisapronil at the present meeting at the request of the Twenty-second Session of CCRVDF (2), with a view to establishing an ADI and recommending MRLs in cattle tissues.

### ***Toxicological and microbiological evaluation***

Information submitted to the Committee included studies on acute, repeated-dose, reproductive and developmental toxicity, genotoxicity and neurotoxicity. Pivotal studies were conducted according to GLP standards and in accordance with OECD and/or VICH guidelines. A literature search provided no additional information.

### ***Biochemical data***

The pharmacokinetics of sisapronil were studied in rats, dogs and cynomolgus monkeys. Most of the investigations were done as part of the toxicological studies.

Twenty-four hours after administration of  $^{14}\text{C}$ -labelled sisapronil by oral gavage to rats at a dose of 50 mg/kg bw, the radioactivity in faeces was approximately 98% of the total recovery from excreta, and the remainder was found in urine, suggesting limited absorption.

An oral bioavailability of 6.8% was determined in cynomolgus monkeys by comparing the pharmacokinetics data in plasma following a single oral administration with those following a single intravenous administration.

There was little information on the distribution and metabolism of sisapronil in laboratory animals. In a radiolabel study in rats, the parent sisapronil accounted for more than 91% of the radioactivity in faeces at 24 hours after oral administration. In addition, sisapronil was identified as the primary residue in the liver. A metabolite was detected in the liver of rats, but could not be identified. It had the same retention time as the main metabolite found in cattle liver, which could not be identified because of the low quantities present. These data suggest that sisapronil undergoes minimal metabolism and that the metabolite found in cattle is also present in the rat and therefore is inherently tested in the toxicity studies on rats.

In rats, the elimination half-life of sisapronil in plasma was approximately 14 days. This half-life was determined in a 28-day repeated-dose toxicity study in which blood samples were taken at 4, 8 and 24 hours post-dosing on several study days, including day 1. The Committee considered that this estimated half-life in rats should be viewed with great caution, because it was based on only three time points and involved a large time extrapolation. The sponsor cited a half-life of 20–39 days, derived from an exploratory oral study in rats with a sampling period of 14 days; however, that study was not provided. The Committee noted



that a time to steady state of approximately 100–150 days could be estimated from the time–concentration profile in plasma in rats from the 1-year repeated-dose toxicity study. In the 1-year rat study, the mean concentrations of sisapronil in plasma at the last time point were approximately 325, 567, 1172 and 3424 ng/mL for the 0.1, 0.3, 1.0 and 10 mg/kg bw per day dose groups, respectively. The increase in sisapronil concentration in plasma was dose dependent, but less than dose proportional. Across the repeated-dose toxicity studies in rats, substantial differences in sisapronil concentrations in plasma were noted, but the cause of this could not be determined.

In a non-GLP-compliant study, dogs were given a single intravenous sisapronil dose of 0.5, 1.5 or 5 mg/kg bw. Blood samples were taken over a period of 268 days. The plasma elimination half-life for all three doses was approximately 100 days.

The concentration of sisapronil in plasma was studied in cynomolgus monkeys over a period of 70 days after a single intravenous administration of 0.5 mg/kg bw. From this study, a plasma elimination half-life of 12.4 days was calculated.

#### *Toxicological data*

Single-dose oral toxicity studies with sisapronil were conducted in mice and rats. The oral LD<sub>50</sub> in rats was 552 mg/kg bw. In mice, no mortality was observed at 30 mg/kg bw, the highest dose tested.

Studies in rabbits showed that sisapronil was not irritating to the skin or the eyes. Sisapronil was not a skin sensitizer in the murine local lymph node assay.

Repeated-dose oral toxicity studies were conducted with sisapronil in rats (gavage) and dogs (capsules). The main studies in rats included a 90-day study and a 1-year study. Studies in dogs included a range-finding 28-day study and a 90-day study.

The main target organs for toxicity in rats and dogs were the liver and thyroid.

In rats given an oral gavage sisapronil dose of 0, 0.1, 0.3, 1.0 or 10 mg/kg bw per day for 90 days, increased levels of thyroid stimulating hormone (TSH) and decreased levels of thyroxine (T<sub>4</sub>) in plasma were observed at the highest dose. Animals of this group also showed increased absolute and relative liver and thyroid weights, hepatocellular hypertrophy and thyroid follicular cell hypertrophy. The NOAEL in this study was 1.0 mg/kg bw per day, based on effects on the liver and thyroid at 10 mg/kg bw per day.

In rats given an oral gavage sisapronil dose of 0, 0.1, 0.3, 1.0 or 10 mg/kg bw per day for 1 year, decreases in levels of triiodothyronine (T<sub>3</sub>) and T<sub>4</sub> in

plasma were observed at the highest dose. Increased absolute and relative liver weights were observed in both sexes at 1.0 and 10 mg/kg bw per day. Increased relative thyroid weights were observed at 1.0 mg/kg bw per day in males only, and absolute and relative thyroid weights were seen at 10 mg/kg bw per day in both sexes. The livers of the animals at 1.0 and 10 mg/kg bw per day showed centrilobular hepatocellular hypertrophy and vacuolation. Thyroid follicular cell hypertrophy, hyperplasia and adenomas were statistically significantly increased at doses of 1.0 and 10 mg/kg bw per day. The NOAEL in this study was 0.3 mg/kg bw per day, based on changes in the liver and thyroid at 1.0 mg/kg bw per day.

In a range-finding 28-day oral toxicity study, dogs were given sisapronil in capsules at a dose of 0, 1, 5 or 25 mg/kg bw per day. Relative liver weights were increased in high-dose males and females, accompanied by increased glycogen in hepatocytes. Absolute and relative thymus weights were decreased in females of the 5 and 25 mg/kg bw per day groups. These decreases were statistically significant in females of the 5 mg/kg bw per day group only. Microscopically, the decreased thymus weights correlated with an increased incidence of minimal to mild decreased cellularity of the lymphoid tissue. The NOAEL in this study was 1 mg/kg bw per day, based on changes in the thymus at 5 mg/kg bw per day.

In a 90-day repeated-dose oral toxicity study, dogs were given sisapronil in capsules at a dose of 0, 0.3, 1 or 10 mg/kg bw per day. Dogs had increased absolute and relative liver weights and vacuolar degeneration of individual hepatocytes at the highest dose. At 1 and 10 mg/kg bw per day, an increase in glycogen in hepatocytes was observed. Minimal thyroid follicular cell hypertrophy was observed at 1 and 10 mg/kg bw per day. Thyroid follicular cell hypertrophy was characterized by cuboidal to tall cuboidal follicular cells, frequently associated with increased vacuolation in the apical cytoplasm. This hypertrophy occurred without increased thyroid weight or changes in thyroid hormone levels in blood. A NOAEL of 0.3 mg/kg bw per day was identified, based on minimal thyroid follicular cell hypertrophy and increased glycogen in hepatocytes at 1 mg/kg bw per day.

The genotoxicity of sisapronil was investigated in an adequate array of *in vitro* and *in vivo* tests. No evidence of genotoxicity was found. *In silico* analysis revealed no structural alerts for genotoxicity. The Committee concluded that sisapronil is not genotoxic.

No specific 2-year toxicity studies or carcinogenicity studies were provided. The study with the longest duration was the 1-year repeated-dose oral toxicity study in rats.

The only test article-related proliferative lesions in the 1-year oral toxicity study in rats described above were thyroid hyperplasia and thyroid tumours (adenomas). The NOAEL for these thyroid effects was 0.3 mg/kg bw per day. A study was conducted to determine a possible mode of action for thyroid hormone

changes and to evaluate the microscopic liver and thyroid changes observed in rats. This study supported a mode of action for sisapronil-induced thyroid tumour formation involving the disruption of homeostasis of the hypothalamic–pituitary–thyroid (HPT) axis by an extrathyroidal mechanism. Specifically, sisapronil induces uridine diphosphate-glucuronosyltransferase (UGT), which increases the conjugation of thyroid hormones ( $T_3$  and  $T_4$ ) in the liver. In response to the hypothyroid state, TSH synthesis and release are stimulated, and this is the key event that leads to thyroid follicular cell growth and hyperplasia. In response to the hypothyroid state and increased TSH levels, the thyroid gland is stimulated to produce more  $T_4$ , and, over time, there is compensatory thyroid gland enlargement. For sisapronil, the available data provide compelling evidence that increased hepatic clearance of  $T_3$  and  $T_4$  causes chronic stimulation of the HPT axis, seen acutely as an increase in TSH secretion, with long-term stimulation of the thyroid gland as a chronic consequence leading to follicular cell adenoma development.

The data from the 1-year rat study were used for benchmark dose (BMD) modelling to assist in the analysis of the proposed mode of action. A number of variables were used for the BMD analysis: TSH, total  $T_4$ , free  $T_3$ , relative (to body and brain weights) liver and thyroid weights as well as the occurrence of bilateral thyroid adenoma and hypertrophy. A benchmark response (BMR) of 10% extra risk above the background was selected as the basis for estimating BMDs. Among the variables analysed, the lower 95% confidence limit on the benchmark dose (BMDL) values ranged from 0.26 to 0.79 mg/kg bw per day, which are supportive of the 0.3 mg/kg bw per day NOAEL identified in this study.

Although the Committee recognized that a specific carcinogenicity study had not been conducted, it concluded that sisapronil is not genotoxic and that the thyroid tumours in rats are a result of an indirect perturbation of the HPT axis by a mode of action not considered relevant to humans. Therefore, the Committee concluded that sisapronil does not pose a risk of carcinogenicity to humans at doses relevant to residues of veterinary drugs.

A two-generation reproductive toxicity study was conducted in rats given sisapronil by oral gavage at a dose of 0, 0.3, 2.0 or 15 mg/kg bw per day. The 15 mg/kg bw per day dose group was discontinued for the  $F_1$  generation because of the high pup mortality in that group. Systemic toxicity was evidenced by significant effects on the liver (increased absolute and relative liver weights) and thyroid (follicular cell hypertrophy and hyperplasia) at 2.0 mg/kg bw per day and higher. Although the reproductive performance of the  $F_0$  generation was not affected at any dose level, low fertility in males and females, decreased male copulation, decreased female conception indices and decreased ovarian follicle counts were noted in the  $F_1$  generation at 2.0 mg/kg bw per day. The NOAEL for reproductive toxicity was 0.3 mg/kg bw per day, the NOAEL for offspring toxicity

was 2.0 mg/kg bw per day and the NOAEL for parental toxicity was 0.3 mg/kg bw per day.

Developmental toxicity studies were conducted in rats and rabbits. The rat study used oral gavage doses of 0, 0.3, 2.0 and 20 mg/kg bw per day. The only effects seen in this study were reduced body weight gain and feed consumption in dams at the high dose, which resulted in lower pup weights in that group. The NOAEL for both maternal toxicity and embryo/fetal toxicity was 2.0 mg/kg bw per day.

The developmental toxicity study in rabbits used oral gavage doses of 0, 0.3, 2.0 and 12.5 mg/kg bw per day. The dams of the high-dose group showed reduced feed intake and severe body weight loss, resulting in moribundity, abortion and premature delivery in several dams. The fetuses of this dose group had lower mean body weights. The NOAEL for both maternal and embryo/fetal toxicity was 2.0 mg/kg bw per day.

The Committee concluded that sisapronil is not teratogenic.

The acute neurotoxicity of sisapronil was investigated in rats given an oral gavage dose of 0, 100, 500 or 1000 mg/kg bw. There was mortality at the highest dose, and animals of the 500 and 1000 mg/kg bw per day groups showed reduced gait, tremors, convulsions (high dose), hyperactivity, vocalization, laboured breathing, reduced foot splay, reduced grip strength and altered reflex responses. Motor activity parameters were also affected in these groups. The NOAEL in this study was 100 mg/kg bw per day.

#### *Microbiological data*

Considering the chemical structure and the mode of action of sisapronil, the Committee did not anticipate any adverse effects of sisapronil residues on the human gastrointestinal microbiota.

#### *Evaluation*

The main findings in the livers of rats and dogs treated with sisapronil included increased liver weights, centrilobular hepatocellular hypertrophy, vacuolation and increased glycogen in hepatocytes. Hepatocellular hypertrophy and increased liver weights in rats are considered to be related to hepatic drug metabolizing enzyme induction, an adaptive response. It is well known that rats are particularly sensitive to developing thyroid tumours according to a mode of action, described above, that is considered not to be relevant to humans.

The Committee noted that the lowest relevant NOAEL was 0.3 mg/kg bw per day in both the 90-day oral toxicity study in dogs and the two-generation reproductive toxicity study in rats. However, the Committee considered this 90-day study in dogs to be unsuitable to address long-term toxicity, in view of the very

long elimination half-life of sisapronil in dogs (i.e. 100 days). Longer-term oral toxicity studies with sisapronil in dogs were not available. It remains uncertain how the toxic effects in dogs might progress and whether or not other effects might be triggered upon longer-term exposure. The Committee also concluded that the NOAEL from the reproductive toxicity study in rats may not be sufficient to protect against long-term effects of sisapronil.

The Committee concluded that a toxicological ADI could not be established because the Committee had no basis upon which to determine a suitable uncertainty factor to accommodate the lack of a long-term toxicity study.

*Additional information that would assist in the further evaluation of the compound*

- Data to address long-term toxicity relevant to humans (e.g. 1-year dog study)
- Comparative pharmacokinetics studies and an explanation of interspecies differences in the pharmacokinetic profiles.

A toxicological monograph was prepared.

**Residue evaluation**

The Committee reviewed studies on the pharmacokinetics and metabolism of sisapronil in cattle, a radiolabelled residue depletion study in cattle, a non-radiolabelled residue depletion study in cattle and information on an analytical method submitted by the sponsor. All studies are compliant with GLP unless otherwise specified.

*Data on pharmacokinetics and metabolism*

**Cattle.** Two non-GLP-compliant studies were conducted examining the pharmacokinetics of non-radiolabelled sisapronil. In one study, cattle were administered a single subcutaneous injection of sisapronil at a dose of 2 mg/kg bw, and sisapronil concentrations were measured in plasma. Sisapronil reached a mean peak concentration of 0.075 µg/mL 15 days post-dose. The pharmacokinetic parameters are a mean observed plasma elimination half-life of 23 days, a mean residence time (MRT) of 48 days and an AUC of 39.5 d·µg/mL.

In the second non-GLP-compliant study, cattle were treated with a single injection of sisapronil at a dose of 2.0 mg/kg bw intravenously or subcutaneously. Following a single intravenous dose, mean clearance of sisapronil was 0.87 L/kg per day, or 0.6 mL/kg per minute, the mean volume of distribution was 24 L/kg and the mean observed plasma elimination half-life was 19 days. Following a single subcutaneous dose, the mean  $C_{\max}$  was 0.072 µg/mL at a mean time to reach  $C_{\max}$  ( $T_{\max}$ ) of 12 days. Based upon comparison of mean AUCs for subcutaneous

and intravenous treatments, the bioavailability after subcutaneous administration was near 100%. The mean observed plasma elimination half-life was 19 days, and the MRT was 32 days.

In a GLP-compliant study, nine groups of three beef cattle each were treated with a single subcutaneous injection of [ $^{14}\text{C}$ ]sisapronil at a dose of 2.0 mg/kg bw, and groups were slaughtered at 10-day intervals from day 10 to day 90 post-dose for tissue residue analysis. Plasma samples were collected from the final two sacrifice groups at study days 1, 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 (final group only) post-dose. Urine and faeces were collected on study days 10–12, 30–32 and 60–62 and analysed for TRR. Parent sisapronil was identified as the primary residue present in fat, liver, kidney and muscle. One significant metabolite accounting for 19–45% of the TRR was observed in liver. Only about 16% of the total dose administered was excreted. Most of the material was excreted via faeces (97%). Radioactivity in plasma peaked at 5 days post-dose, reaching a mean of 343  $\mu\text{g eq/kg}$  (80 days post-dose group) and 300  $\mu\text{g eq/kg}$  (90 days post-dose group). Total radioactivity in plasma declined to a mean of 42  $\mu\text{g eq/kg}$  at 80 days post-dose and 26  $\mu\text{g eq/kg}$  at 90 days post-dose.

#### *Residue data*

**Cattle.** One study using [ $^{14}\text{C}$ ]sisapronil was performed. Cattle were treated with a single subcutaneous dose of [ $^{14}\text{C}$ ]sisapronil of 2.0 mg/kg bw, and groups of four animals (two of each sex) were slaughtered at 10-day intervals from day 10 to day 90 post-dose. Total radioactivity was analysed, and sisapronil residues were determined. In edible tissues, TRR were highest in fat, followed by liver, kidney and then loin muscle. Mean TRR in fat were 10 195  $\mu\text{g eq/kg}$  at 30 days, declining to 891  $\mu\text{g eq/kg}$  at 90 days. Mean TRR in liver were 2793  $\mu\text{g eq/kg}$  at 10 days, declining to 299  $\mu\text{g eq/kg}$  at 90 days. Mean TRR in kidney were 1552  $\mu\text{g eq/kg}$  at 10 days, declining to 133  $\mu\text{g eq/kg}$  at 90 days. Mean TRR in hindquarter muscle were 183  $\mu\text{g eq/kg}$  at 10 days, declining to below the LOD at 90 days.

Based on the data from this study, the Committee took a conservative approach and chose the lowest value reported over all time points analysed. The following MR:TRR ratios were established for the following tissues: 0.90 for muscle, 0.50 for liver, 0.86 for kidney and 0.94 for fat.

In another study, the depletion of non-radiolabelled sisapronil was studied using cattle that received a single subcutaneous injection of sisapronil of 2.0 mg/kg bw. Groups of four animals (two of each sex) were slaughtered at 30-day intervals from 30 to 240 days post-dose, and sisapronil concentrations were determined using an LC-MS/MS method with an LOQ of 5  $\mu\text{g/kg}$ . In edible tissues, at all time points, the highest residue concentrations were observed in fat, followed by liver, kidney and then hindquarter muscle, with results above the

LOQ observed at all time points. Mean residues in fat were 7520 µg/kg at 30 days, declining to 564 µg/kg at 240 days. Mean residues in liver were 759 µg/kg at 30 days, declining to 60 µg/kg at 240 days. Mean residues in kidney were 465 µg/kg at 30 days, declining to 43 µg/kg at 240 days. Mean residues in hindquarter muscle were 172 µg/kg at 30 days, declining to 32 µg/kg at 240 days. Mean residues in core injection site muscle were 29 650 µg/kg at 30 days, declining to 76 µg/kg at 240 days. Mean residues in surrounding injection site were 4007 µg/kg at 30 days, declining to 119 µg/kg at 240 days.

The Committee noted that the residue depletion data suggested that the terminal elimination phase was appreciably longer than the reported plasma elimination half-life.

#### *Analytical methods*

An LC-MS/MS method was used to determine the marker residue (parent sisapronil) in bovine edible tissues. The marker residue is extracted by solid-liquid extraction. After agitation and centrifugation, the supernatant is transferred to an HPLC vial. The stationary phase was a C18 column, and the mobile phase was 0.027% formic acid in 2 mM ammonium acetate (volume per volume [v/v]) (A) and acetonitrile (B). Sisapronil residues are ionized in an electrospray interface (negative polarity) before acquisition of the signal in the selected reaction monitoring mode in a triple quadrupole mass spectrometer. Sisapronil labelled at three positions ( $^{13}\text{C}_2$ - $^{15}\text{N}$ ) was used as an internal standard. Standard curves were generated using simple linear regression.

The Committee assessed the validation data against the requirements for analytical methods as published in CAC/GL 71-2009 (15). The method has been validated for selectivity, precision, accuracy, LOD and LOQ. No interfering peaks were observed at the retention time of sisapronil in any of the non-fortified samples, attesting to the selectivity of the method. The intra-day and inter-day mean accuracies were in the range 85–110% and 94–105%, respectively, for all tissues. The intra-day and inter-day precisions were in the range 1.1–17.5% and 3.4–12.4%, respectively, for all tissues. The calculated LODs were 0.2 µg/kg for muscle, 0.6 µg/kg for liver, 0.6 µg/kg for kidney and 0.3 µg/kg for fat. The calculated LOQs were approximately 0.6 µg/kg for muscle, 1.6 µg/kg for liver, 1.7 µg/kg for kidney and 0.8 µg/kg for fat; the method validation established 5 µg/kg as the LOQ for sisapronil in bovine edible tissues. The analytical method proposed for routine residue surveillance is a gradient LC-MS/MS method applicable in the range of 5–1000 µg/kg for all tissues. Accordingly, the method is expected to be practicable and applicable in normal routine laboratory use.



### Maximum residue limits

MRLs could not be recommended by the Committee, as an ADI could not be established.

A residue monograph was prepared.

### Summary and conclusions

#### Studies relevant to risk assessment

Species / study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
<b>Rat</b>				
Three-month study of toxicity (gavage)	0, 0.1, 0.3, 1.0, 10	Hepatocellular hypertrophy; thyroid follicular cell hypertrophy	1.0	10
One-year study of toxicity (gavage)	0, 0.1, 0.3, 1.0, 10	Centrilobular hepatocellular hypertrophy; thyroid follicular cell hypertrophy, hyperplasia and adenoma	0.3	1.0
Two-generation reproductive toxicity study (gavage)	0, 0.3, 2.0, 15	Parental toxicity: Follicular cell hypertrophy and hyperplasia; increased liver weights	0.3	2.0
		Reproductive toxicity: Low fertility in males and females, decreased male copulation, decreased female conception indices and decreased ovarian follicle counts in F <sub>1</sub> generation	0.3	2.0
		Offspring toxicity: Decreased survival	2.0	15
Developmental toxicity study (gavage)	0, 0.3, 2.0, 20	Embryo/fetal toxicity: Lower pup weight due to lower body weight gain and lower feed consumption in dams	2.0	20
<b>Rabbit</b>				
Developmental toxicity study (gavage)	0, 0.3, 2.0, 12.5	Maternal toxicity: Severe body weight loss, reduced feed intake, moribundity, abortion and premature delivery	2.0	12.5
		Embryo/fetal toxicity: Reduced pup weight	2.0	12.5
<b>Dog</b>				
Three-month study of toxicity <sup>a</sup> (capsule)	0, 0.3, 1, 10	Increased glycogen in hepatocytes; thyroid follicular cell hypertrophy	0.3	1

<sup>a</sup> A plasma half-life of approximately 100 days was found in a non-GLP-compliant study in dogs.

### ADI

No ADI was established because the Committee had no basis upon which to determine a suitable uncertainty factor to accommodate the lack of a long-term toxicity study.



## MRLs

MRLs could not be recommended by the Committee, as an ADI could not be established.

## 4.4 Teflubenzuron

### **Explanation**

Teflubenzuron (IUPAC name: 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea; CAS no. 83121-18-0) is an insecticide belonging to the benzoylurea group of compounds. Its mode of action is through inhibition of the synthesis of chitin, and hence it is most effective on the developmental stages of insects. Insects are killed as a result of the disruption of their moulting. Teflubenzuron is approved in many countries as an insecticide for use in plant production as well as for control of sea lice (*Lepeophtheirus salmonis* and *Caligus rogercresseyi*) in aquaculture. Teflubenzuron is used as a premix coated onto non-medicated fish feed pellets to achieve an intended dose of 10 mg/kg bw per day for 7 consecutive days. The withdrawal periods range from 7 to 11 days and from 45 to 96 degree-days.

Teflubenzuron has not been previously evaluated by the Committee, although it was evaluated by JMPR as a pesticide in 1994 and 1996 (18, 19) and is scheduled for periodic re-evaluation at JMPR's September 2016 meeting. JMPR established an ADI of 0–0.01 mg/kg bw on the basis of a LOAEL of 2.1 mg/kg bw per day in a mouse carcinogenicity study with the application of an uncertainty factor of 200, including an additional factor of 2 to account for the use of a LOAEL instead of a NOAEL.

The Committee evaluated teflubenzuron at the present meeting at the request of the Twenty-second Session of the Codex Committee on Residues of Veterinary Drugs in Foods (2), with a view to establishing an ADI and recommending MRLs in finfish tissues.

### **Toxicological and microbiological evaluation**

The Committee considered data on the pharmacokinetics, short- and long-term toxicity, reproductive and developmental toxicity, genotoxicity and carcinogenicity of teflubenzuron. In addition to a sponsor's submission, relevant studies retrieved from the published literature were evaluated. Most studies submitted by the sponsor were conducted under GLP-compliant conditions. Those that were not conducted under GLP-compliant conditions are identified in this report.

### *Biochemical data*

Orally administered teflubenzuron was only partially, but relatively quickly, absorbed. Following a single oral dose of radiolabelled teflubenzuron at 25 mg/kg bw, approximately 20% of the radioactivity was absorbed; only 4% was absorbed when rats were dosed at 750 mg/kg bw, suggesting a dose-dependent absorption. Peak plasma concentrations were reached within 1–2 hours post-dosing and were maintained at similar levels for up to 8 hours (low dose) or 24 hours (high dose). In repeatedly dosed animals, there was some evidence of a dose-dependent plateau in plasma concentration.

Most (90–95%) radiolabelled teflubenzuron administered by gavage to rats (single dose at 25 or 750 mg/kg bw or 14 daily doses of 25 mg/kg bw of unlabelled drug followed by a single dose of 25 mg/kg bw radiolabelled drug) was excreted in faeces, primarily as the parent compound. More than 85% of the drug was excreted within the first 24 hours of the dosing. Only a small fraction (0.15–3%) of the total oral dose of teflubenzuron was excreted in the urine. There was no difference in excretion pattern between sexes or between animals dosed with a single or multiple doses of the drug. Absorbed teflubenzuron was mostly excreted through bile, predominantly as polar materials. Only negligible residues of teflubenzuron were detected in tissues and organs (<2% of the dose), with no evidence of accumulation.

Metabolites identified in bile and urine were benzoyl or aniline ring hydroxylated teflubenzuron and conjugates of (3,5-dichloro-2,4-difluorophenyl)-urea and 3,5-dichloro-2,4-difluoroaniline. Several polar metabolites were detected in faeces, but the only metabolite characterized was (3,5-dichloro-2,4-difluorophenyl)urea. Hydrolytic cleavage of the phenylurea bridge was identified as the predominant pathway of teflubenzuron metabolism in a non-GLP-compliant study in which rats were gavaged once with approximately 55 mg/kg bw of the drug. The scission products thus produced were either excreted unmodified or further metabolized and excreted.

### *Toxicological data*

Teflubenzuron was shown to have low acute toxicity in laboratory animals. The oral LD<sub>50</sub> in mice and rats was greater than 5000 mg/kg bw. The dermal LD<sub>50</sub> in rats was greater than 2000 mg/kg bw, and the inhalation LC<sub>50</sub> in rats was greater than 5000 mg/m<sup>3</sup> air. Teflubenzuron was not irritating to the skin or eyes of rabbits, and it did not cause skin sensitization in the guinea-pig maximization test.

Short-term toxicity studies of teflubenzuron in which the drug was administered in diet were conducted in mice (one 13-week study), rats (one 13-week study) and dogs (two 13-week studies and one 52-week study). In all three

species, liver was identified as the target organ for toxic effects, as evidenced by elevated enzyme activities and/or adaptive cellular changes.

In a study whose GLP-compliant status could not be verified, mice were administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg diet (equal to 0, 12, 115 and 1213 mg/kg bw per day for males and 0, 14, 142 and 1450 mg/kg bw per day for females, respectively) for 13 weeks. In the high-dose group, activities of liver enzymes (alkaline phosphatase in males, alanine transaminase in females) and total cholesterol levels (females) were elevated, and blood glucose levels (both sexes) were altered. In the mid- and high-dose groups, absolute and relative liver weights were increased, with centrilobular hepatocellular swelling (both sexes) and microscopic fatty changes (in males). The NOAEL was identified as 100 mg/kg feed (equal to 12 mg/kg bw per day), based on liver changes at 1000 mg/kg feed (equal to 115 mg/kg bw per day).

Rats were administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 8, 82 and 809 mg/kg bw per day for males and 0, 9, 94 and 942 mg/kg bw per day for females, respectively) for 13 weeks. Some animals from the control and high-dose groups were observed for an additional 4 weeks without treatment. Increased activities of several enzymes, notably aspartate transaminase (middle and high doses, both sexes), ornithine transcarbamylase (high dose, both sexes), alanine transaminase (mid- and high-dose males), lactate dehydrogenase (high-dose males) and alkaline phosphatase (all treated males), were observed, which returned to normal levels after 4 weeks of drug withdrawal. At necropsy, increased absolute and relative weights of liver in females and of testes in males were observed at the high dose. The statistically significant change in alkaline phosphatase activity in male rats of the low-dose group was not considered to be biologically relevant, being less than 1.5-fold above the control value and within the normal physiological range. Based on effects on liver enzymes at 1000 mg/kg feed (equal to 82 mg/kg bw per day), a NOAEL of 100 mg/kg feed (equal to 8 mg/kg bw per day) was identified.

In a non-GLP-compliant study, the potential of teflubenzuron to form methaemoglobin and exert other effects on haematological parameters in rats was investigated in a comparative study that investigated five benzoylurea insecticides. Although reticulocyte count was increased in rats treated with teflubenzuron (100 mg/kg bw per day for 28 days), it was not associated with anaemia or the formation of methaemoglobin.

In dogs administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 3.5, 33.7 and 318.2 mg/kg bw per day for males and 0, 4.0, 42.8 and 417.1 mg/kg bw per day for females, respectively) for 13 weeks, activities of alanine transaminase, aspartate transaminase, alkaline phosphatase and ornithine transcarbamylase were increased in both sexes at the high dose. At necropsy, absolute and relative liver weights were elevated and the incidence of

nodular foci in the pyloric or fundic region of the stomach was increased at the high dose. Isolated dark red foci were noted in the pyloric region of the stomach at the middle and high doses. Focal gastritis was observed in females at the middle and high doses, and follicular hyperplasia of the pyloric mucosa was noted in most animals at the high dose. Mild hepatitis in one male and centrilobular hepatic necrosis in another male were observed at the low dose. Also, moderate chronic active hepatitis was diagnosed in one animal of each sex at the high dose. Given the lack of a clear dose–response relationship for hepatitis, the mild microscopic hepatic changes noted in two dogs in the low-dose group were not considered to be a treatment-related adverse effect. The NOAEL was 100 mg/kg feed (equal to 3.5 mg/kg bw per day), based on stomach lesions at 1000 mg/kg feed (equal to 33.7 mg/kg bw per day).

In a supplementary study to clarify the effects on liver, dogs were administered teflubenzuron at a concentration of 0, 30 or 100 mg/kg feed (equal to 0, 1.2 and 4.4 mg/kg bw per day for males and 0, 1.5 and 5.1 mg/kg bw per day for females, respectively) for 13 weeks. There were no treatment-related adverse effects identified in clinical examination, laboratory testing, and macroscopic or microscopic examination of organs. The NOAEL was 100 mg/kg feed (equal to 4.4 mg/kg bw per day), the highest dose tested.

In a 52-week study, dogs were administered teflubenzuron at a concentration of 0, 30, 100 or 500 mg/kg feed (equal to 0, 1.0, 3.2 and 17.3 mg/kg bw per day for males and 0, 1.2, 4.0 and 18.0 mg/kg bw per day for females, respectively). At necropsy, the absolute liver weight in males was increased at the high dose, but no treatment-related changes were noted in gross pathological or histopathological examinations. The NOAEL was 100 mg/kg feed (equal to 3.2 mg/kg bw per day), based on the liver weight change at 500 mg/kg feed (equal to 17.3 mg/kg bw per day).

The Committee identified an overall NOAEL of 100 mg/kg feed (equal to 4.4 mg/kg bw per day) from three short-term studies in dogs, based on the findings of adverse effects in liver at a dose of 500 mg/kg feed (equal to 17.3 mg/kg bw per day).

In a carcinogenicity study in mice (20), teflubenzuron was administered at a concentration of 0, 15, 75 or 375 mg/kg feed (equal to 0, 2.1, 10.5 and 53.6 mg/kg bw per day for males and 0, 3.1, 15.4 and 71.7 mg/kg bw per day for females, respectively) for 78 weeks, with an interim kill at week 52. Aspartate transaminase, alanine transaminase, ornithine transcarbamylase, lactate dehydrogenase and alkaline phosphatase activities were elevated in high-dose males, but only alanine transaminase activity was elevated in high-dose females. Absolute and relative liver weights were higher in both sexes at the high dose, and relative liver weight was slightly increased in the mid-dose males. The incidence of macroscopic hepatic nodules was increased in the high-

dose males. Histopathology indicated an increased incidence of hepatocellular adenomas and nodular hepatic hyperplasia in males treated at the middle and high doses compared with both concurrent and historical controls, but there was no difference in the incidence of hepatic carcinoma. Several treatment-related, dose-dependent, non-neoplastic hepatic changes were also observed, which were more pronounced in males than in females. In particular, males in the control, low-dose, mid-dose and high-dose groups, respectively, had dose-dependent incidences of hepatocellular hypertrophy (12/60, 29/60, 46/60 and 56/60), single-cell necrosis (13/60, 26/60, 42/60 and 56/60), phagocytic cell foci (17/60, 21/60, 43/60 and 54/60) and lipofuscin accumulation (8/60, 11/60, 20/60 and 27/60). In the low-dose group, the incidence, but not the severity, of these non-neoplastic hepatic changes was significantly higher when compared with the controls.

Histopathological sections of liver from male mice in this study were re-evaluated by an independent pathologist, with a focus on nodular liver lesions. The pathologist concluded that there was a dose-related increase in the incidence of hepatocellular hyperplastic nodules and a slight, but statistically non-significant, increase in hepatocellular adenoma.

Given that only hepatic adenomas were observed and that the genotoxicity test results were negative (see below), the Committee considered that teflubenzuron was not carcinogenic in mice. However, the Committee concluded that teflubenzuron induced hyperplastic proliferation in liver of mice by an unknown mechanism. Based on the increased incidence of non-neoplastic hepatic changes observed in liver (e.g. hepatocellular hypertrophy, single-cell necrosis, phagocytic cell foci, lipofuscin accumulation) at all doses, no NOAEL could be identified. The lowest dietary concentration, 15 mg/kg feed (equal to 2.1 mg/kg bw per day), was identified as the LOAEL.

In the absence of a NOAEL, to better characterize the point of departure, the Committee conducted a dose–response analysis of these data using the BMD approach. Of several non-neoplastic hepatic changes identified, hepatocellular hypertrophy was considered to be the most toxicologically relevant effect for dose–response modelling. The BMD and BMDL for a 10% response over the controls (BMD<sub>10</sub> and BMDL<sub>10</sub>) were determined using nine different dichotomous models. Three models (LogLogistic, LogProbit and Multistage) provided acceptable fits based on statistical considerations. However, the BMD<sub>10</sub> and BMDL<sub>10</sub> estimated by the LogLogistic and LogProbit models were much lower than the lowest dose used in the study. Furthermore, the Multistage model provided a better fit of the BMDL value for the benchmark response at the low end of the observed range of the data. Therefore, the Committee considered the BMDL<sub>10</sub> of 0.54 mg/kg bw per day for the BMD<sub>10</sub> of 0.73 mg/kg bw per day estimated by the Multistage model as the most appropriate point of departure for this study.

In a carcinogenicity study, rats were administered teflubenzuron at a concentration of 0, 20, 100 or 500 mg/kg feed (equal to 0, 1.0, 4.8 and 24.8 mg/kg bw per day for males and 0, 1.2, 5.9 and 29.9 mg/kg bw per day for females, respectively) for 120 weeks, with an interim kill at weeks 53 and 107. Mortality ranged from 40% to 50% at week 120, which was not influenced by treatment. Increased (approximately 1.5- to 3-fold) activities of alanine transaminase, aspartate transaminase and ornithine transcarbamylase were noted in males in the high-dose group. Absolute and relative liver weights were increased in high-dose males. Several non-neoplastic microscopic changes were noted in different organs, but were not treatment related. Trend analysis identified increased incidences of haemangiomas in mesenteric lymph nodes and pancreatic exocrine carcinoma in the high-dose males. However, they were not significantly different when compared with historical controls. Also, the occurrence of pancreatic exocrine carcinoma was too infrequent (2/47 versus 0/50) to allow a meaningful comparison to be drawn. Based on the effect on liver enzymes and liver weight at 500 mg/kg feed (equal to 24.8 mg/kg bw per day), the NOAEL was 100 mg/kg feed (equal to 4.8 mg/kg bw per day).

In a supplemental carcinogenicity study, rats were administered teflubenzuron at a concentration of 0, 2500 or 10 000 mg/kg feed (equal to 0, 123 and 487 mg/kg bw per day for males and 0, 154 and 615 mg/kg bw per day for females, respectively) for 111 weeks, with an interim kill at week 104. Clinical biochemistry revealed increased activities of alanine transaminase and aspartate transaminase in males at both doses and of aspartate transaminase in females at the high dose. The absolute and relative liver (interim and terminal kill) and kidney (interim kill) weights were increased in males at the high dose compared with controls. Dose-dependent increases in the incidence of diffuse, clay-coloured discoloration and focal and multifocal discoloration of livers were observed in treated males (both doses). Also, treatment-related non-neoplastic microscopic changes (e.g. fatty changes, mixed cell and basophilic cell foci, focal hepatocellular hyperplasia, spongiosis hepatitis) were noted in the liver of both sexes at both doses tested, lesions being more severe in males than in females. There was no compound-related increase in the incidence of any tumours observed in this study, including mesenteric lymph node haemangioma and pancreatic exocrine carcinoma in male rats, thus confirming the lack of association between substance administration and the occurrence of these tumours suggested from the previous study.

Although no NOAEL could be identified in the second study owing to non-neoplastic microscopic hepatic changes and elevated liver enzyme activities in both treatment groups, the Committee was able to identify an overall NOAEL of 100 mg/kg feed (equal to 4.8 mg/kg bw per day) from the two chronic toxicity and carcinogenicity studies in rats.

The Committee concluded that teflubenzuron is not carcinogenic in mice or rats.

The genotoxic potential of teflubenzuron was investigated in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was detected, and teflubenzuron was considered unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Committee concluded that teflubenzuron is unlikely to pose a carcinogenic risk to humans.

In a multigeneration reproductive toxicity study, teflubenzuron was administered to rats at a concentration of 0, 20, 100 or 500 mg/kg feed (equal to 0, 1.5, 7.4 and 36.9 mg/kg bw per day for males and 0, 1.6, 7.9 and 39.5 mg/kg bw per day for females, respectively). The only treatment-related adverse effect noted was a significant increase in the incidence of unilateral and bilateral dilatation of the renal pelvis in F<sub>1</sub> pups in the high-dose group (6.8%) when compared with the controls (0.9%). No such effect was seen in the F<sub>2</sub> generation. The NOAEL for offspring toxicity was 100 mg/kg feed (equal to 7.4 mg/kg bw per day), and the NOAEL for both parental and reproductive toxicity was 500 mg/kg feed (equal to 36.9 mg/kg bw per day), the highest dose tested.

The developmental toxicity of teflubenzuron was investigated in pregnant rats by gavage administration at 0, 10, 50 or 250 mg/kg bw per day from days 6 to 15 of gestation. The number of live pups per dam was significantly reduced at the high dose when compared with the controls. The NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested, and the NOAEL for embryo/fetal toxicity was 50 mg/kg bw per day, based on a reduction in the number of live pups per dam at 250 mg/kg bw per day.

In a second developmental toxicity study, teflubenzuron was administered by gavage to pregnant rats at 0, 100, 300 or 1000 mg/kg bw per day during days 7–17 of gestation. No treatment-related toxicity was observed in dams, for both general health and reproductive parameters. No external, visceral or skeletal abnormalities were observed in pups. The NOAEL for both maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

An overall NOAEL of 1000 mg/kg bw per day for maternal toxicity was identified. No overall NOAEL for embryo/fetal toxicity could be identified, as the reason for the difference in NOAELs for embryo/fetal toxicity in these two developmental toxicity studies in rats was unknown.

Pregnant rabbits were dosed with teflubenzuron by gavage at 0, 10, 50 or 250 mg/kg bw per day from days 6 to 18 of gestation and killed on day 29 of gestation. No maternal or reproductive toxicity was observed, and there were no developmental abnormalities. The only significant effect noted in the offspring was decreased survival during the first 24 hours in the high-dose group (88.5%) compared with the controls (100%). The NOAEL for embryo/fetal toxicity was



50 mg/kg bw per day, based on decreased survival at 250 mg/kg bw per day, and the NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested.

To further elucidate the embryotoxic effect in rabbits, a small supplementary study (five per group) was conducted by administering teflubenzuron by gavage at 0, 250, 500 (killed on day 19) or 500 mg/kg bw per day (killed at day 29) on days 6–18 of gestation. There was evidence of embryotoxicity in all treated animals, although no maternal toxicity was identified.

In another developmental toxicity study in rabbits, pregnant animals were dosed with teflubenzuron by gavage at 0 or 1000 mg/kg bw per day during days 6–18 of pregnancy and killed on gestation day 28. Treated rabbits had a higher incidence of liver lesions compared with controls, but there were no treatment-related reproductive or developmental abnormalities. No NOAEL was identified for maternal toxicity, as effects were noted at the only dose tested. The NOAEL for embryo/fetal toxicity was 1000 mg/kg bw per day, the only dose tested. However, this study did not evaluate offspring survival during the first 24 hours, and hence the Committee cannot discount the effects seen in the previous study.

An overall NOAEL of 500 mg/kg bw per day was identified for maternal toxicity, and an overall NOAEL of 50 mg/kg bw per day was identified for embryo/fetal toxicity.

#### *Microbiological data*

Considering the chemical structure and mode of action of teflubenzuron, the Committee did not anticipate any adverse effects of teflubenzuron residues on human gastrointestinal microbiota.

#### *Evaluation*

An ADI of 0–5 µg/kg bw was established on the basis of a BMDL<sub>10</sub> of 0.54 mg/kg bw per day for hepatocellular hypertrophy in male mice observed in the carcinogenicity study, with application of an uncertainty factor of 100 to account for interspecies and intraspecies variability, and rounded to one significant figure.

The use profile of teflubenzuron as a veterinary drug is such that dietary exposure to teflubenzuron from a large portion is unlikely to be markedly greater than that from chronic consumption. The toxicological profile of teflubenzuron is such that it is unlikely to present an acute hazard. The Committee therefore concluded that it was not necessary to assess the acute risk from exposure to teflubenzuron when used as a veterinary drug.

A toxicological monograph was prepared.



### **Residue evaluation**

The Committee reviewed studies on the pharmacokinetics and metabolism of teflubenzuron in Atlantic salmon. Also, a number of radiolabelled and non-radiolabelled teflubenzuron residue depletion studies in Atlantic salmon were reviewed. The analytical method submitted by the sponsor to support the residue monitoring has been assessed.

All studies were GLP compliant unless otherwise stated.

#### *Data on pharmacokinetics and metabolism*

Two studies evaluated the pharmacokinetics of teflubenzuron in Atlantic salmon held at a water temperature of 13–14 °C. Plasma samples were taken at 15 minutes and 3, 6, 9, 12, 24, 48, 72, 120 and 168 hours post-treatment.

In the first study, Atlantic salmon weighing 173–395 g received a single dose of 2 mg/kg bw teflubenzuron by intravenous injection. At 15 minutes after dosing, teflubenzuron was detected in plasma at a concentration of  $5.2 \pm 4.9$  µg/mL (mean  $\pm$  SD; 10 fish). The pharmacokinetic parameters were:  $AUC_{(0-72)}$  of 23.4 µg·h/mL, half-life ( $t_{1/2}$ ) of 15.3 hours and clearance (CL) of 1.4 mL/kg per minute.

In the second study, Atlantic salmon weighing 100–425 g received a single dose of teflubenzuron by gavage at a dose of 10 mg/kg bw. The maximum concentration of teflubenzuron in plasma was  $0.5 \pm 0.1$  µg/mL (mean  $\pm$  SD; 10 fish) at 9 hours post-dose. The pharmacokinetic parameters were:  $T_{max}$  of 9.0 hours,  $AUC_{(0-72)}$  of 10.9 µg·h/mL and  $t_{1/2}$  of 14.2 hours.

In another study, Atlantic salmon weighing 626–918 g were held at a water temperature of 7–8 °C and received teflubenzuron medicated feed at a daily dose of 10 mg/kg bw for 7 consecutive days. Plasma samples were collected during the feeding period (6 hours post-feeding on days 1–7) and after 30 and 48 hours following administration of the last medicated feed. The maximum teflubenzuron concentration in plasma was  $0.250 \pm 0.074$  µg/mL on day 6 during the administration of the medicated feed. Thirty hours after the last dose, the concentration of teflubenzuron had depleted to  $0.10 \pm 0.065$  µg/mL.

Two metabolism studies were conducted with Atlantic salmon at a water temperature of 10 °C. In the first study, Atlantic salmon weighing 537–999 g received [<sup>14</sup>C]teflubenzuron at a single dose of 10 mg/kg bw by intra-oesophageal intubation. Tissue samples were collected at 9 hours and 1, 3, 4, 6, 8, 13 and 18 days post-dose. TRR was determined at 1 and 8 days post-dose. The marker residue, teflubenzuron, was the major residue in muscle on day 1 (MR:TRR ratio 0.99) and day 8 (MR:TRR ratio 0.84) post-dosing. In skin, the MR:TRR ratio was 1.04 and 0.77 at day 1 and day 8, respectively. In liver, 3,5-dichloro-2,4-difluoroaniline (3.1% of TRR) and 3'-hydroxy-teflubenzuron (3.3% of TRR) were detected at 1 day post-dosing. One metabolite (6.4% of TRR) remained unidentified in liver.

In the second study, Atlantic salmon (508–1297 g) received medicated feed at a teflubenzuron dose of 10 mg/kg bw for 6 consecutive days. On the seventh day, the fish received [<sup>14</sup>C]teflubenzuron at a dose of 10 mg/kg bw by intra-oesophageal intubation. On day 1 post-dose, the major residue in salmon tissues (muscle, skin, liver and kidney) was the parent compound, determined by radio-HPLC. In liver, as in the first study, 3,5-dichloro-2,4-difluoroaniline and 3'-hydroxy-teflubenzuron were identified. The MR:TRR ratio at day 8 post-dose was 1.28 in muscle and 0.82 in skin.

Based on the results of these two studies, the Committee determined that a value of 0.8 was appropriate for the MR:TRR ratio. This value was the mean value of the MR:TRR ratio of muscle and skin determined 8 days post last dose of teflubenzuron at a water temperature of 10 °C, excluding the value of 1.28, which was considered an outlier.

#### *Residue data*

Three residue depletion studies using radiolabelled teflubenzuron and two residue depletion studies using non-radiolabelled teflubenzuron were provided for Atlantic salmon, using a single oral dose or repeated dose at two water temperatures (6 °C and 10 °C). Teflubenzuron was quantified in salmon tissues using a validated HPLC-UV method. Average recoveries were above 70%, and the LOQ of the method was 20 µg/kg for muscle and skin in natural proportion.

In one study, Atlantic salmon (537–999 g), held at a water temperature of 10 °C, received a dose of 10 mg/kg bw of [<sup>14</sup>C]teflubenzuron by intra-oesophageal intubation. Six fish were sampled at each of the following intervals: 9 hours and 1, 3, 4, 6, 8, 13 and 18 days post-dose. Tissues (mucus, liver, kidney, muscle, skin and gallbladder) were collected, and the TRR was determined using liquid scintillation spectrometry. The acetonitrile-extractable residue on day 8 post-dose accounted for 84% (muscle), 77% (skin), 54% (liver) and 54% (kidney) of the TRR. The highest concentration of radioactivity in muscle ( $410 \pm 89.0$  µg eq/kg) and skin ( $753 \pm 224$  µg eq/kg) was determined 1 day after administration of the drug.

In a similar depletion study, Atlantic salmon maintained at 10 °C received non-radiolabelled teflubenzuron in medicated feed, at a dose of 10 mg/kg bw, for 6 consecutive days; on day 7, the fish received a dose of [<sup>14</sup>C]teflubenzuron of 10 mg/kg bw by intra-oesophageal intubation. Only a small amount of radioactive material was distributed into the tissues examined – that is, the majority of material was excreted from the fish. The highest quantity of radioactive material was detected in the muscle ( $310 \pm 124$  µg eq/kg) and skin ( $554 \pm 178$  µg eq/kg) 1 day after administration of the last dose.

In another study, Atlantic salmon (527–1403 g), held at a water temperature of 6 °C, received non-radiolabelled teflubenzuron in the diet for 13

days at a dose of 10 mg/kg bw; on the 14th day, the fish received a dose equivalent to 10 mg/kg bw of [ $^{14}\text{C}$ ]teflubenzuron by intra-oesophageal intubation. Tissues were collected 1, 8, 16, 24, 35, 50, 75 and 97 days post-treatment. Liver contained the highest concentration of radioactive material, with a maximum of  $1170 \pm 336 \mu\text{g eq/kg}$  on day 1, which decreased with an elimination half-life of 16.9 days determined over the period of 1–24 days. For muscle and skin, the maximum concentrations occurred 1 day following the final dose ( $153 \pm 40 \mu\text{g eq/kg}$  for muscle and  $218 \pm 83 \mu\text{g eq/kg}$  for skin).

The last two studies using non-radiolabelled teflubenzuron were conducted at two water temperatures (6 °C and 10 °C). For the low temperature, Atlantic salmon received teflubenzuron in the diet for 13 days; on the 14th day, the fish were treated with the same dose of teflubenzuron by intra-oesophageal intubation. Tissues were collected and analysed 1, 8, 16, 24, 35 days post-treatment. The concentration of teflubenzuron in muscle with skin depleted from  $407 \mu\text{g/kg}$  (day 1) to  $25 \mu\text{g/kg}$  (day 35). For the high water temperature, Atlantic salmon received 10 mg/kg bw teflubenzuron in the diet over a 7-day period. One fish per group received a single oral dose of 10 mg/kg bw teflubenzuron by intra-oesophageal intubation on feeding day 7. Samples of muscle and skin were collected on days 1, 4, 8, 12, 18, 24, 35, 50 and 120 post-dose. The concentration of teflubenzuron in muscle with skin depleted from  $931 \mu\text{g/kg}$  (day 1) to  $38 \mu\text{g/kg}$  (day 35). The half-life of elimination calculated from the residue data from days 1 to 18 in the combined muscle and skin was 3.4 days.

Teflubenzuron residues depleted in muscle and skin with different half-lives depending on the water temperature. Peak residue concentrations were higher in the experiment performed at 10 °C than in the experiment at 6 °C; however, the initial rates of depletion of tissue residues were similar. The slow terminal phase of elimination was attributed to background levels of teflubenzuron in the recirculated seawater in the tanks where the fish were housed. Consequently, the data from time points 24 and 35 days were not used. Only the time points 1, 4, 8, 12 and 18 days were used to determine the MRL. The radiolabel studies indicated that the main residue in muscle and skin is the parent compound and that the excretion of teflubenzuron is predominantly via faeces.

### *Analytical methods*

The Committee assessed the validation data against the requirements for analytical methods as published in CAC/GL 71-2009 (15).

The validated HPLC-UV method with detection at 254 nm is based on solid-liquid extraction, followed by several clean-up steps using liquid-liquid extraction and solid-phase extraction on two sorbents (silica and C8). Quantification was performed using an external calibration curve in the

concentration range of 0.02–1.0 µg/mL (corresponding to 20–1000 µg/kg). The linearity was 0.9950, and recoveries were above 70%. The LOQ was 20 µg/kg of teflubenzuron in salmon muscle and skin in natural proportion. The Committee concluded that the HPLC-UV method provided lacks in selectivity because of possible interferences from other components in the extract at a wavelength of 254 nm and cannot be recommended for regulatory monitoring of salmon tissues for teflubenzuron.

The Committee noted that some national authorities monitor teflubenzuron in salmon tissues using a multi-residue pesticide monitoring procedure that may be applicable for regulatory monitoring of salmon tissues. The sample preparation is a modification of the QuEChERS approach without using the dispersive sample clean-up step. Briefly, salmon tissue is shaken with a mixture of water and acetonitrile in the presence of sodium chloride and magnesium sulfate. The mixture is centrifuged, and a volume of the extract is added to ammonium formate and formic acid. The filtered extract is analysed by LC-MS/MS, using the electrospray ionization source in the negative mode. The chromatographic separation is carried out on a reversed-phase C18 column and a mobile phase of ammonium formate and formic acid under gradient elution. The precursor ion at 379 *m/z* and two product ions at 339.1 *m/z* and 358.9 *m/z* are used for identification and quantification. Concentrations in salmon muscle and skin were determined by external calibration curves (standards in acetonitrile), without using an internal standard.

The validation parameters reported were: linear range of 0.1–100 ng/mL corresponding to 0.3–300 µg/kg, linearity of 0.9995, intraday precision of 1.64–6.02% (1–100 µg/kg), extraction recovery in the range of 65.9–101.4% (1–100 µg/kg) and an estimated LOQ (signal to noise  $\geq 10$ ) of 0.3 µg/kg. The estimated LOD, which is defined as the standard concentration that can be detected with signal to noise ratio  $\geq 3$ , is 0.03 ng/mL (equivalent to 0.09 µg/kg). The selectivity of the method was demonstrated, and incurred samples were also used in the validation procedure. The Committee considered that the method used by the national authorities and published in the literature could be recommended for regulatory monitoring of salmon tissues for teflubenzuron.

#### *Maximum residue limits*

In recommending MRLs for teflubenzuron in salmon, the Committee considered the following factors:

- Teflubenzuron is authorized for use in salmon in several countries. The maximum recommended dose is 10 mg/kg fish per day for 7 consecutive days, administered through medicated feed. The withdrawal periods range from 7 to 11 days and from 45 to 96 degree-days.

- An ADI for teflubenzuron of 0–5 µg/kg bw was established by the Committee.
- Teflubenzuron is the marker residue in tissues.
- The ratio of the concentration of marker residue to the concentration of total residue of 0.8 was calculated in muscle and skin in natural proportion of salmon. Residue data were provided using a validated analytical method to quantify teflubenzuron in salmon tissues.
- A validated analytical method for the determination of teflubenzuron in edible salmon tissues is available in the literature and may be used for monitoring purposes.

The MRLs were calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile of total residue concentrations (95/95 UTL) in salmon muscle and skin derived from the pivotal study used for this assessment, conducted at a water temperature of 10 °C and a withdrawal period of 96 degree-days (10 days).

The Committee recommended MRLs for teflubenzuron in salmon of 400 µg/kg in fillet (muscle plus skin in natural proportion) and in muscle.

The EDI is 42.9 µg/person per day, on the basis of a 60 kg individual, which represents approximately 14% of the upper bound of the ADI.

The GECDE for the general population is 1.6 µg/kg bw per day, which represents 31% of the upper bound of the ADI; for children, 2.1 µg/kg bw per day, which represents 43% of the upper bound of the ADI; and for infants, 0.9 µg/kg bw per day, which represents 18% of the upper bound of the ADI.

A residue monograph was prepared.

## Summary and conclusions

### Studies relevant to risk assessment

Species / study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
<b>Mouse</b>				
Eighteen-month carcinogenicity study (diet)	0, 2.1, 10.5, 53.6	Hepatocellular adenoma	2.1	10.5
		Hepatocellular hypertrophy	–	2.1 <sup>a</sup>
			BMDL <sub>10</sub> : 0.54 <sup>a</sup>	–
<b>Rat</b>				
Two-year toxicity and carcinogenicity study (diet)	0, 1.0, 4.8, 24.8	Increased activity of liver enzymes, and increased liver weights in males	4.8 <sup>b</sup>	24.8 <sup>c</sup>
Two-generation reproductive toxicity study (diet)	0, 1.5, 7.4, 36.9	Unilateral and bilateral dilatation of the renal pelvis in F <sub>1</sub> pups	Offspring toxicity: 7.4	36.9
			Parental toxicity: –	36.9 <sup>d</sup>

Species / study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Developmental toxicity studies <sup>c</sup> (gavage)	Study 1: 0, 10, 50, 250 Study 2: 0, 100, 300, 1 000	Decreased number of live pups per dam	Reproductive toxicity: 36.9 <sup>d</sup>	–
			Embryo/ fetal toxicity: 50 (study 1)	250 (study 1)
			Maternal toxicity: 1 000 <sup>b,d</sup>	–
<b>Rabbit</b>				
Developmental toxicity studies <sup>c</sup> (gavage)	Study 1: 0, 10, 50, 250 Study 2: 0, 250, 500 Study 3: 0, 1 000	Decreased survival of offspring within 24 h of birth	Embryo/fetal toxicity: 50 <sup>b</sup>	250 <sup>c</sup>
			Maternal toxicity: 500 <sup>b,d</sup>	–
<b>Dog</b>				
Thirteen-week studies of toxicity <sup>e</sup> (diet)	Study 1: 0, 3.5, 33.7, 318.2 Study 2: 0, 1.2, 4.4	Increased liver weight	4.4 <sup>b</sup>	17.3 <sup>c</sup>
One-year study of toxicity (diet)	0, 1.0, 3.2, 17.3			

\* Pivotal study value for the derivation of an ADI (20)

<sup>a</sup> Lowest dose tested.

<sup>b</sup> Overall NOAEL.

<sup>c</sup> Overall LOAEL.

<sup>d</sup> Highest dose tested.

<sup>e</sup> Two or more studies combined.

### Uncertainty factor

100 (10 for interspecies and 10 for intraspecies variability)

### ADI (based on toxicological effects)

0–5 µg/kg bw

### Residue definition

Teflubenzuron

### MRLs

The recommended MRL for teflubenzuron in salmon fillet (muscle plus skin in natural proportion) and in salmon muscle is 400 µg/kg.

### Estimated dietary exposure

The EDI is 42.9 µg/person per day, on the basis of a 60 kg individual, which represents approximately 14% of the upper bound of the ADI. The GECDE for the general population is 1.6 µg/kg bw per day, which represents 31% of the upper

bound of the ADI; for children, 2.1 µg/kg bw per day, which represents 43% of the upper bound of the ADI and for infants, 0.9 µg/kg bw per day, which represents 18% of the upper bound of the ADI.

## 4.5 Zilpaterol hydrochloride

### **Explanation**

Zilpaterol hydrochloride, ( $\pm$ )-*trans*-4,5,6,7-tetrahydro-7-hydroxy-6-(isopropylamino)imidazo[4,5,1-jk]-[1]benzazepin-2(1*H*)-one hydrochloride (zilpaterol HCl; CAS no. 119520-06-8), is a  $\beta_2$ -adrenoreceptor agonist repartitioning agent. It is used to increase rate of body weight gain, improve feed efficiency and increase carcass muscle ratio in cattle fed in confinement before slaughter. There are four enantiomers of zilpaterol HCl. The product in use is racemic *trans*-zilpaterol HCl, a mixture of the (6*R*,7*R*) and (6*S*,7*S*) enantiomers; it will be referred to as zilpaterol HCl in this report.

Zilpaterol HCl is to be mixed into the feed at a level of 7.5 mg/kg on a 90% dry matter basis. This will result in a dose of approximately 0.15 mg/kg bw, or 60–90 mg zilpaterol HCl per animal per day. Zilpaterol HCl is administered for a period of 20–40 consecutive days before withdrawal from the feed. It is not approved for use in lactating dairy cattle. Where information on authorized uses was provided, withdrawal periods ranged from 2 to 4 days.

The seventy-eighth meeting of the Committee, at the request of the Twenty-first Session of CCRVDF (6), evaluated zilpaterol HCl and established an ADI of 0–0.04 µg/kg bw on the basis of a LOAEL for a slight increase of tremor in humans in a single-dose study ([Annex 1](#), reference 217). The seventy-eighth Committee concluded that it was not possible to recommend MRLs for zilpaterol and that the following data were needed to establish MRLs:

- results from studies investigating marker residue in liver and kidney;
- results from studies determining the MR:TRR ratio in liver and kidney;
- results from depletion studies to enable the derivation of MRLs compatible with the ADI.

Following the submission of additional information, the present Committee evaluated the data to recommend MRLs for bovine tissues. In addition, at the request of the Twenty-second Session of CCRVDF (2), JECFA considered potential risks of zilpaterol residues in animal lungs and other edible offal. In line with evolving guidance on the need to consider the establishment of ARfDs for veterinary drugs, the Committee evaluated the acute risk of zilpaterol.

In addition, the Committee addressed a number of comments received from the sponsor on the evaluation of zilpaterol HCl by the seventy-eighth JECFA.

### *Toxicological evaluation*

No new toxicological data for zilpaterol HCl were submitted by the sponsor, except for information on the structure–activity relationship of *N*-acetylated deisopropyl zilpaterol.

### *Previous JECFA evaluation*

The Committee at its seventy-eighth meeting considered the acute pharmacological effect observed in four human studies, which was consistent with the agonistic activity of zilpaterol HCl on the  $\beta_2$ -adrenoceptor, to be the most sensitive adverse effect for the establishment of an ADI for zilpaterol HCl. The LOAEL for a slight and transitory increased incidence of tremor was 0.76  $\mu\text{g}/\text{kg}$  bw, the lowest dose tested, which was found in fasted humans who ingested a single oral dose of zilpaterol HCl as a bolus (21). The Committee established an ADI of 0–0.04  $\mu\text{g}/\text{kg}$  bw by applying an uncertainty factor of 20, comprising a default uncertainty factor of 10 for human individual variability and an additional uncertainty factor of 2 to account for the use of a LOAEL for a slight effect instead of a NOAEL (Annex 1, reference 217). Despite the fact that the ADI was based on an acute effect, the seventy-eighth JECFA did not consider establishing an ARfD, as this was not normal practice at the time.

### *Bioavailability of incurred residues*

In the data submission for the current meeting, the sponsor provided comments suggesting that the bioavailability of the incurred residues needs to be considered in the determination of total residues of concern for zilpaterol HCl because the design of the human studies did not reflect realistic exposure of the consumer to possible zilpaterol residues in food. The sponsor asserted that the pharmacological effects resulting from incurred zilpaterol are reduced by a factor of 10 in comparison with an oral bolus administration. This assertion is based on a study in dogs showing that administration of zilpaterol HCl as an incurred residue at a dose of 2  $\mu\text{g}/\text{kg}$  bw did not cause any effects, but the same dose administered orally in a capsule induced a slight increase in heart rate; and a pharmacokinetics study in rats in which the  $C_{\text{max}}$  values were 10 times higher in serum when zilpaterol HCl was administered via oral gavage in comparison with a dietary admixture.

The Committee reviewed the sponsor's comments and concluded as follows:



- There were no changes in blood pressure or heart rate in dogs exposed to zilpaterol HCl at approximately 2 µg/kg bw per day for 5 days in the form of incurred residues in 200 g of muscle or liver from steers. In contrast, exposure of fasted dogs to zilpaterol HCl elicited an 11% increase in heart rate when the zilpaterol HCl was administered via capsule at 2 µg/kg bw and an increase of 11.8% when the zilpaterol HCl was administered via capsule at 3 µg/kg bw together with 200 g of muscle or liver from untreated steers (22). These data provide some indication that the pharmacological potency of zilpaterol HCl is dependent on the bioavailability of incurred residues. However, this study was inconclusive, as only small numbers of animals were used (one male and one female per group). Additional limitations include the absence of an oral (gavage) study in non-fasted dogs and the absence of human fed/fasted, dietary/oral dose comparative data.
- The pharmacokinetics of zilpaterol HCl were investigated in male and female rats following administration by dietary admixture or oral gavage for a 2-week period at a dose of 0.055 or 1.1 mg/kg bw per day (23). The  $C_{\max}$  for zilpaterol HCl administered by oral admixture was 8.5–15.7% of that following oral gavage administration. However, the AUC following administration by oral admixture was 38.8–105.7% of that following oral gavage administration. These data suggest that zilpaterol administration via dietary admixture may result in prolonged absorption and lower peak plasma concentrations, but does not clearly lead to lower bioavailability. However, there was considerable variation in the  $T_{\max}$ ,  $C_{\max}$  and AUC values observed in this study, making it difficult to reach clear conclusions on the differences between the pharmacokinetics of zilpaterol residues administered as a bolus dose or dietary admixture.

### *Pharmacological potency*

The present Committee reviewed the pharmacological potency of metabolites of zilpaterol HCl. The sponsor submitted a new structure–activity relationship assessment of *N*-acetylated deisopropyl zilpaterol to make the case that this metabolite has no pharmacological activity. The Committee concluded as follows:

- The affinity of zilpaterol HCl for the rat lung  $\beta_2$ -adrenoceptor in vitro was about 1.5-fold higher than that of its main metabolite, deisopropyl zilpaterol (as free base or hydrochloride form) (24). However, three in vivo rat studies demonstrated that zilpaterol HCl displays 10-fold higher  $\beta_2$ -adrenoceptor agonist activity compared with its main

metabolite, deisopropyl zilpaterol (25–27). The Committee confirmed its previous view that the potency of deisopropyl zilpaterol was 10% of that of the parent.

- Another metabolite, *N*-acetylated deisopropyl zilpaterol, found in rat urine at up to 4% of the administered dose, is predicted to have no pharmacological activity based on an assessment of its structure–activity relationship (28). The Committee agreed with this assessment.
- Multi-step metabolism of zilpaterol is likely to lead to disruption of the pharmacophore for  $\beta_2$ -adrenoceptor agonist activity, and therefore the pharmacological potency of such metabolites will be less than that of the parent compound. A conservative estimate for the pharmacological potency of such unidentified polar extractable residues would be 10% of that of the parent compound (similar to the potency of the metabolite deisopropyl zilpaterol).

#### *Assessment of acute effects*

The present Committee noted that the basis of the previously established ADI was an acute effect in humans after a single dose of zilpaterol HCl. In line with evolving guidance on the need to consider the establishment of ARfDs for veterinary drugs, the Committee considered it appropriate to establish an ARfD. The acute agonistic effect on  $\beta_2$ -adrenoceptor in humans (21) was the most sensitive effect observed and therefore serves as the basis for both the ADI and the ARfD.

#### *Evaluation*

The present Committee reaffirmed the ADI of 0–0.04  $\mu\text{g}/\text{kg}$  bw that was established at the seventy-eighth meeting of JECFA and established an ARfD of 0.04  $\mu\text{g}/\text{kg}$  bw based on a LOAEL of 0.76  $\mu\text{g}/\text{kg}$  bw for acute pharmacological effects observed in the single-dose human study, with application of an uncertainty factor of 20, comprising a default uncertainty factor of 10 for human individual variability and an additional uncertainty factor of 2 to account for use of a LOAEL for a slight effect instead of a NOAEL.

An addendum to the toxicological monograph was not prepared.

#### *Residue evaluation*

The sponsor submitted additional data that included results from two new depletion studies in cattle, a validated analytical method for residues in bovine tissues and an assessment of the pharmacological impact of residues in the exposure assessment. The studies were compliant with GLP unless otherwise stated.

*Pharmacokinetics and residue data*

The seventy-eighth Committee agreed that parent zilpaterol (free base) was an appropriate marker residue in muscle. Only limited data were available for tissues other than muscle, and the Committee was unable to determine a suitable marker residue in other edible tissues. Liver and kidney contained the highest concentrations of zilpaterol at all sampling times, followed by muscle. The zilpaterol concentrations, and the subsequent ratios of zilpaterol to the total residue of concern, could not be determined with confidence over the 96-hour withdrawal period after the last drug administration due to the limited data available and lack of analytical method sensitivity. There are no measurable residues in fat. Pharmacokinetics, radiolabelled residue studies and the earlier non-radiolabelled residue studies were already assessed by the seventy-eighth JECFA. The previous evaluation by the Committee noted that zilpaterol is readily absorbed after oral administration, although the degree of absorption may vary depending on the specific method of oral dosing. In a rat study using the Gallo-Torres model, the bioavailability of non-extractable radiolabelled zilpaterol residues from cattle liver was  $\leq 5\%$  (29). Metabolism studies conducted in rats, swine and cattle demonstrated the metabolism of zilpaterol to be qualitatively and quantitatively comparable in these three species following oral administration. Metabolism is mainly via deisopropylation and hydroxylation pathways. Hepatic metabolism in cattle produces deisopropyl zilpaterol and its *N*-acetyl product. Hydroxy-zilpaterol and its glucuronide conjugates are produced in rats, but have not been detected in cattle tissues. Deisopropyl zilpaterol was the only metabolite accounting for more than 10% of the radioactivity found in edible tissues of cattle. Parent compound and metabolites are excreted primarily (>85%) in the urine, with the remainder in the faeces. Unchanged parent zilpaterol is the main compound excreted in the urine. No new pharmacokinetics data were received for this meeting.

The previous evaluation by the Committee noted that radiolabelled residue depletion studies were conducted in cattle after treatment at the recommended dose of 0.15 mg/kg bw per day. The radiolabel data from a limited number of animals provided a depletion curve for a period limited to 96 hours post-dose. No residues were detected in fat after 12 hours, and no residues were detected in muscle after 48 hours. Residues were detected in liver and kidney at 96 hours post-dose. Extractable residues from liver decreased from 52% to 24% of total radioactivity between 12 and 96 hours and from 89% to 38% for kidney over the same period. Residues in muscle were approximately 100% extractable between 12 and 48 hours. Parent zilpaterol (free base) represented a significant part of the extractable residue in liver, kidney and muscle. The ratio of parent zilpaterol to total residues decreased with time for liver, kidney and muscle.

Deisopropyl zilpaterol comprised a minor fraction of the extractable residue. Other metabolites detected in small quantities in cattle include *N*-acetylated deisopropyl zilpaterol (urine only) and one unidentified metabolite. Non-radiolabelled zilpaterol residue depletion studies were also provided, although numerous deficiencies were noted in some studies. These included lack of kidney residue determination in all but one study, insufficient sampling periods and insufficiently sensitive analytical methods.

*New residue data submitted to the current Committee*

Data from the two new residue depletion studies provided by the sponsor were evaluated by the present Committee, together with data from previously evaluated residue depletion studies. The two new studies provided a more robust estimate of residue depletion in the main target tissues, liver, kidney and muscle, due to use of improved analytical methods and a longer sampling period (up to 240 hours post-dose). Such data provided confirmation of the marker residue in all relevant tissues. Residue data were provided for 60 and 90 mg/head per day dose groups, but only data from the upper limit of the approved dose range (90 mg/head per day) were used in the subsequent exposure assessment. Furthermore, residue depletion modelling of a pooled data set combined from multiple studies was considered but deemed inappropriate due to significant differences in the study methods.

From the radiolabel study, the total pharmacologically active residue (expressed as zilpaterol HCl equivalents) was calculated using the following formula:

$$\text{Total pharmacologically active residue} = \text{Zilpaterol HCl} + 0.1 \cdot [\text{RR}_{\text{Ext}} + (0.05 \cdot \text{RR}_{\text{NonExt}})]$$

where:

- Zilpaterol HCl = parent zilpaterol concentration, expressed as zilpaterol hydrochloride;
- 0.1 = relative pharmacological activity correction factor. The activity attributed to zilpaterol HCl was set as 1, whereas the activity of all other extractable and bioavailable non-extractable residues was set as 0.1 (i.e. 10% of the parent zilpaterol activity);
- $\text{RR}_{\text{Ext}}$  = sum of other extractable radioactive residue concentrations (including the major metabolite deisopropyl zilpaterol), expressed as zilpaterol HCl-eq;
- $\text{RR}_{\text{NonExt}}$  = non-extractable (bound) radioactive residue concentration, expressed as zilpaterol HCl-eq;

- 0.05 = oral bioavailability of non-extractable residues (as per the Gallo-Torres model).

The ratios ( $R_{\text{tissue}(t)}$ ) between parent zilpaterol and total pharmacologically active residue (based on the radiolabel study, as calculated above) over time were determined. Ratios in liver decreased from 94% to 74% between 12 and 96 hours and from 99% to 50% for kidney over the same period. For muscle, the ratio decreased from 92% to 84% between 12 and 48 hours.

The concentrations of zilpaterol free base obtained in the pivotal non-radiolabelled residue depletion study were converted into total pharmacologically active residue (expressed as zilpaterol HCl-eq) using the following formula:

$$\text{Total pharmacologically active residue} = 1.1395 * [\text{Zilpaterol free base}] / R_{\text{tissue}(t)}$$

where:

- 1.1395 = molecular weight conversion factor, required to convert all zilpaterol free base residues to zilpaterol HCl for comparisons with the ADI;
- Zilpaterol free base = marker residue concentration;
- $R_{\text{tissue}(t)}$  = ratio between marker residue and total pharmacologically active residue estimated at an equivalent time point ( $t$ ) for each tissue (liver, kidney, muscle) from the radioactive residue study.

#### *Dietary exposure assessment*

Both chronic and acute dietary exposures were considered for residues of zilpaterol. As the ADI and ARfD for zilpaterol are based on an acute pharmacological endpoint, the most relevant approach was deemed the acute exposure assessment. For acute exposure, the GEADE approach was used. Large portion size values based on the 97.5th percentile of food consumption were used in the GEADE assessment of zilpaterol. The consumption amounts used as inputs were based on data from more than 70 consumers to ensure that acute exposure estimates were statistically robust.

#### *Analytical method*

A new method was used for the analysis of free zilpaterol residues in the pivotal studies. In brief, samples of homogenized bovine tissue were fortified with an internal standard ( $[^2\text{H}_7]$ zilpaterol) and extracted with methanol. A sub-sample of the extract was purified by cation exchange solid-phase extraction and then analysed by a validated LC-MS/MS method using electrospray ionization in the

positive ion mode and selected reaction monitoring as the acquisition method. Quantification was performed using a solvent calibration curve corresponding to a range of 0.25–30 µg/kg tissue equivalents for all tissues. The Committee assessed the validation data against the requirements for analytical methods as published in CAC/GL 71-2009 (15). For all tissues, the LOQ was 0.25 µg/kg. Calculated LODs were 0.048, 0.067 and 0.045 µg/kg for liver, muscle and kidney, respectively. The average recovery of zilpaterol in the methanol extracts was determined to be 76% (liver), 85% (kidney) and 73% (muscle). The confirmatory method proposed for routine residue surveillance is a gradient LC-MS/MS method applicable in the range of 0.25–30 µg/kg for all tissues. Accordingly, the method is expected to be practicable and applicable in normal routine laboratory use.

#### *Maximum residue limits*

In recommending MRLs for zilpaterol, the Committee considered the following factors:

- An ARfD of 0.04 µg/kg bw was established. This is the same value as the upper bound of the ADI previously established by the seventy-eighth Committee and reaffirmed by the present Committee.
- Zilpaterol HCl is registered to be mixed into feed at a level of 7.5 mg/kg on a 90% dry matter basis. This level provides a dose of approximately 0.15 mg/kg bw or 60–90 mg zilpaterol HCl per animal per day.
- Where information on authorized uses was provided, withdrawal periods ranged from 2 to 4 days.
- Zilpaterol HCl is not approved for use in lactating dairy cattle.
- The major metabolite in cattle tissues is deisopropyl zilpaterol.
- Zilpaterol HCl administration in cattle results in non-extractable residues that are poorly bioavailable in laboratory animals. This low oral bioavailability (~5%) demonstrated for liver was assumed to be similar for non-extractable residues in muscle and kidney.
- The most sensitive toxicological end-point is an acute pharmacological effect. It was assumed that zilpaterol HCl has a reference activity of 1. Deisopropyl zilpaterol was shown to have ~10% of the pharmacological activity of parent zilpaterol, with the activity of all other extractable and bioavailable non-extractable residues being equivalent to, or less than, that of deisopropyl zilpaterol.
- Parent zilpaterol (free base) was an appropriate marker residue in muscle, liver and kidney. Fat was not considered relevant for residue monitoring purposes.

- The ratios of zilpaterol (MR) to the total residues of concern (total pharmacologically active residues) for muscle, liver and kidney could be determined with sufficient confidence over a 96-hour period after the last drug administration. The MR:total pharmacologically active residue ratios were between 0.9 and 1.0 for liver, kidney and muscle at 12 hours withdrawal. By 96 hours withdrawal, the MR:total pharmacologically active residue ratios were 0.7 (liver and muscle) and 0.5 (kidney).
- A validated analytical procedure for the determination of zilpaterol in edible bovine tissues (liver, kidney, muscle) is available and may be used for monitoring purposes. The LOQ is 0.25 µg/kg for all tissues.

The MRLs recommended for bovine tissues are based on an acute dietary exposure scenario (GEADE). The Committee initially derived the following one-sided 95% confidence interval over the 95th percentile of residue concentrations (95/95 UTL) in bovine tissues at the 72-hour time point: 4.1 µg/kg in kidney, 4.3 µg/kg in liver and 0.6 µg/kg in muscle. Using acute dietary exposure assessments (GEADE), these 95/95 UTLs could lead to an acute dietary exposure of ~99% of the ARfD in the general population and ~117% of the ARfD in children.

Because the exposure in children exceeded the ARfD using the 72-hour data, the Committee considered a refined assessment with 95/95 UTLs derived at 77 hours post-dose: 3.3 µg/kg in kidney, 3.5 µg/kg in liver and 0.5 µg/kg in muscle. These values would result in acute dietary exposure (GEADE of 1.9 µg/day for the general population and 0.57 µg/day for children) of ~94% of the ARfD in children and ~80% of the ARfD in the general population and are recommended as MRLs. It is noted that the time point at which the MRLs are calculated (77 hours) is consistent with currently approved withdrawal times (GVP).

The Committee recognizes that the approach used in this evaluation differs from that of previous evaluations for similar types of veterinary compounds. However, this was appropriate due to the acute nature of the pharmacological end-point and the availability of an appropriate model for acute exposure. Detailed chronic and acute dietary exposure assessments are included in the addendum to the residue monograph to provide additional information to risk managers.

The Committee concluded that there were insufficient zilpaterol residue data to adequately consider exposure to residues in lungs and other edible offal of cattle apart from liver and kidney. No non-radiolabelled residue depletion data were provided for any cattle tissues other than liver, kidney and muscle. For lung tissue, there were no actual residue data available in cattle, just estimates based on ratios of plasma versus respiratory tissue radioactivity from preliminary radiolabel studies in rats. For edible offal, the only bovine data available were

from a preliminary radiolabel study, with only two data points for tripe at each of the 12- and 48-hour withdrawal periods.

#### *Recommendation*

The Committee noted that the definitions of the tissues comprising offal were not consistent between countries. Therefore, JECFA requests that CCRVDF provide a definition of edible offal.

An addendum to the residue monograph was prepared.

#### **Summary and conclusions**

##### *Uncertainty factor (for both ADI and ARfD)*

20 (10 for intraspecies variability, 2 for use of a LOAEL instead of a NOAEL)

##### *ADI*

0–0.04 µg/kg bw

##### *ARfD*

0.04 µg/kg bw

##### *MRLs*

The recommended MRLs for cattle are 3.3 µg/kg in kidney, 3.5 µg/kg in liver and 0.5 µg/kg in muscle.

##### *Estimated dietary exposure*

GEADEs of 1.9 µg/day for the general population and 0.57 µg/day for children were calculated, based on 95/95 UTLs, which represent approximately 80% and 94% of the upper bound of the ARfD for the general population and children, respectively.

##### *Comments from the sponsor*

- a. The sponsor identified several errors in some of the tables in the seventy-eighth JECFA monograph, which it believed may have had an impact on data interpretation and conclusions.
- b. The sponsor stated that data gaps identified by the seventy-eighth JECFA were not fully justified, as available information in submitted studies had not been used by the Committee.



- c. The sponsor stated that there were sufficient data sets (including the new studies – not available at the time of the seventy-eighth JECFA) to recommend MRLs.

**Response from JECFA:** The corrected tables have been included where necessary in the eighty-first JECFA addendum to the residue monograph. Assessment of the data has been performed using an approach based on all data available.

- d. The sponsor stated that only the residues of pharmacological concern are relevant for the dietary exposure assessment, as the ADI was based on a pharmacological end-point. In particular, the sponsor argued that insufficient attention was paid to the 10-fold difference in activity between zilpaterol and its main metabolite (deisopropyl zilpaterol) with respect to  $\beta_2$ -agonist activity on the cardiovascular system.

**Response from JECFA:** The evaluation reflects this comment.

- e. Regarding residues of pharmacological concern, the sponsor proposed that the reduced bioavailability of zilpaterol residues (and thus not pharmacologically active) should be accounted for in the exposure assessment.

**Response from JECFA:** The bioavailability of the non-extractable portion of incurred bound residues was considered in the assessment, as per the Gallo-Torres model. A bioavailability correction factor of 0.05 was used for all non-extractable residues. All extractable residues were assumed to be fully bioavailable, as per current regulatory guidance in multiple jurisdictions, and the available data do not support the sponsor's proposal.



## 5. Future work and recommendations

Recommendations relating to specific veterinary drugs, including ADIs, ARfDs and proposed MRLs, are given in [section 3](#) and [Annex 2](#). This section includes recommendations relating to future work by the JECFA Secretariat.

### Diflubenzuron

*Additional information that would assist in the further evaluation of the compound*

- A comparative metabolism study of diflubenzuron in humans and rats (e.g. in hepatocytes)
- Information on PCA exposure associated with the consumption of treated fish
- Information on the amount of PCA present (if any) as an impurity in the product formulation
- Information on the amount of PCA generated during food processing
- A method suitable for monitoring diflubenzuron residues in fish muscle and fillet (muscle plus skin in natural proportion).

### *Recommendation*

The Committee recommends that JMPR consider the re-evaluation of diflubenzuron at a future meeting and that the WHO Pesticide Evaluation Scheme (WHOPES) and the WHO *Guidelines for Drinking-water Quality* (GDWQ) Chemical Working Group reconsider their recommendations for the use of diflubenzuron as a vector control agent in drinking-water.

### Sisapronil

*Additional information that would assist in the further evaluation of the compound*

- Data to address long-term toxicity relevant to humans (e.g. 1-year dog study)
- Comparative pharmacokinetic studies and an explanation of interspecies differences in the pharmacokinetic profiles.

### Zilpaterol hydrochloride

The Committee noted that the definitions of the tissues comprising offal were not consistent between countries. Therefore, JECFA requests that CCRVDF provide a definition of edible offal.

### **Acute reference dose (ARfD) for veterinary drugs**

The Committee recommends that a subgroup be established to review available information on acute exposure to residues of veterinary drugs and to identify an upper-bound exposure value with sufficient confidence that will enable, if possible, the derivation of a cut-off value for acute toxicity.

### **Chronic dietary exposure assessment**

The Committee recommends that the FAO and WHO Secretariats convene an expert meeting on two important issues concerned with the methodologies applied by JECFA and JMPR to estimate chronic dietary exposures.

*In regards to dietary exposure assessment of compounds used for multiple purposes (i.e. veterinary drugs and pesticides):*

1. Develop chronic dietary exposure assessment methods that take into account combined exposure from pesticide and veterinary drug residues.
2. Investigate the applicability of these methods using compounds that have been evaluated as both pesticides and veterinary drugs.

*In regards to dietary exposure assessment for less-than-lifetime exposure:*

1. Investigate the effects of duration of exposure in toxicity studies on veterinary drugs on toxicological end-points and the points of departure (e.g. NOAELs).
2. Based on the outcome of #1, identify those toxicological situations requiring less-than-lifetime exposure assessment.

*In regards to dietary exposure assessment*

1. Apply the methodologies developed above to some key examples of veterinary drugs and pesticides that are unlikely to accumulate (including compounds that have been evaluated as both pesticides and veterinary drugs) and report the outcome to JECFA and JMPR.

### **Coordination of the agendas of JECFA and JMPR**

The Committee strongly recommends that where dual-use substances are to be evaluated by both JMPR and JECFA, CCPR and CCRVDF coordinate the prioritization of such substances for evaluation by the respective experts. The Committee also recommends that the Joint Secretariats of JMPR and JECFA ensure that there is suitable interaction between experts in the evaluation of such compounds.

## **Acknowledgements**

The Committee wishes to thank Ms M. Sheffer, Ottawa, Canada, for her assistance in the preparation of the report.

FAO and WHO wish to acknowledge the significant contributions of the experts, as well as their institutions (where relevant), to the work of the eighty-first meeting of JECFA.



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## Annex 1

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219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.
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221. Safety evaluation of certain food additives. WHO Food Additives Series, No. 70, 2015.
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## Annex 2

### Recommendations on compounds on the agenda

#### Diflubenzuron (insecticide)

- Acceptable daily intake** In the absence of adequate information on exposure to 4-chloroaniline (PCA), a genotoxic and carcinogenic metabolite and/or degradate of diflubenzuron, the Committee was unable to establish an acceptable daily intake (ADI) for diflubenzuron because it was not possible to assure itself that there would be an adequate margin of safety from its use as a veterinary drug. The Committee also noted that it was not possible to calculate a margin of exposure for PCA in the absence of adequate information on exposure to PCA.
- Maximum residue limits** The Committee was unable to recommend maximum residue limits (MRLs) for diflubenzuron, as an ADI could not be established

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#### Ivermectin (antiparasitic agent)

- Acceptable daily intake** The Committee established an ADI of 0–10 µg/kg body weight on the basis of a no-observed-adverse-effect level (NOAEL) of 0.5 mg/kg body weight per day for neurological effects (mydriasis) and retardation of weight gain in a 14-week dog study, with application of an uncertainty factor of 50 (5 for interspecies differences based on pharmacokinetics studies in dogs and humans and 10 for intraspecies differences). The previous ADI of 0–1 µg/kg body weight was withdrawn.
- Acute reference dose** The Committee established an acute reference dose (ARfD) of 0.2 mg/kg body weight, based on a NOAEL of 1.5 mg/kg body weight, the highest dose tested in a safety, tolerability and pharmacokinetics study in healthy human subjects, with application of an uncertainty factor of 10 for intraspecies variability.

Estimated chronic dietary exposure	<p>The estimated daily intake (EDI) is 38 µg/person per day, based on a 60 kg individual, which represents 6% of the upper bound of the ADI.</p> <p>The global estimate of chronic dietary exposure (GECDE) for the general population is 0.9 µg/kg body weight per day, which represents 9% of the upper bound of the ADI.</p> <p>The GECDE for children is 1.5 µg/kg body weight per day, which represents 15% of the upper bound of the ADI.</p> <p>The GECDE for infants is 1.3 µg/kg body weight per day, which represents 13% of the upper bound of the ADI.</p>
Estimated acute dietary exposure	<p>The maximum values of residues found at injection sites led to global estimates of acute dietary exposure (GEADE) of 73 µg/kg body weight for the general population and 82 µg/kg body weight for children, corresponding, respectively, to 36% and 41% of the ARfD.</p>
Residue definition	Ivermectin B <sub>1a</sub>

**Recommended maximum residue limits (MRLs)<sup>a</sup>**

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	400	100	800	30

<sup>a</sup> No new data were provided for use of ivermectin in dairy cattle; therefore, the Committee did not recommend any revision to the MRL of 10 µg/kg for ivermectin in milk.

**Lasalocid sodium** (antiparasitic agent)

Following consideration of the issues raised in concern forms from the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), the Committee concluded that there would be no concern for colonization barrier disruption in the colon from acute exposure to residues of lasalocid. The ADI established and MRLs recommended at the seventy-eighth meeting of JECFA (WHO TRS No. 988, 2014) remain unchanged.



**Sisapronil (ectoparasiticide)**

Acceptable daily intake	The Committee concluded that a toxicological ADI could not be established because the Committee had no basis upon which to determine a suitable uncertainty factor to accommodate the lack of a long-term toxicity study.
Maximum residue limits	The Committee could not recommend MRLs, as an ADI could not be established.

**Teflubenzuron (insecticide)**

Acceptable daily intake	The Committee established an ADI of 0–5 µg/kg body weight on the basis of a lower 95% confidence limit on the benchmark dose for a 10% response (BMDL <sub>10</sub> ) of 0.54 mg/kg body weight per day for hepatocellular hypertrophy in male mice observed in a carcinogenicity study, with application of an uncertainty factor of 100 to account for interspecies and intraspecies variability.
Estimated chronic dietary exposure	<p>The EDI is 42.9 µg/person per day, on the basis of a 60 kg individual, which represents approximately 14% of the upper bound of the ADI.</p> <p>The GECDE for the general population is 1.6 µg/kg body weight per day, which represents 31% of the upper bound of the ADI.</p> <p>The GECDE for children is 2.1 µg/kg body weight per day, which represents 43% of the upper bound of the ADI.</p> <p>The GECDE for infants is 0.9 µg/kg body weight per day, which represents 18% of the upper bound of the ADI.</p>
Residue definition	Teflubenzuron

**Recommended maximum residue limits (MRLs)**

Species	Fillet <sup>a</sup> (µg/kg)	Muscle (µg/kg)
Salmon	400	400

<sup>a</sup> Muscle plus skin in natural proportion.

**Zilpaterol hydrochloride** (β<sub>2</sub>-adrenoceptor agonist)

- Acceptable daily intake    The Committee reaffirmed the ADI of 0–0.04 µg/kg body weight established at the seventy-eighth meeting (WHO TRS No. 988, 2014).
- Acute reference dose        The Committee established an ARfD of 0.04 µg/kg body weight based on a lowest-observed-adverse-effect level (LOAEL) of 0.76 µg/kg body weight for acute pharmacological effects observed in a single-dose human study, with application of an uncertainty factor of 20, comprising a default uncertainty factor of 10 for human individual variability and an additional uncertainty factor of 2 to account for use of a LOAEL for a slight effect instead of a NOAEL.
- Residue definition         Zilpaterol (free base) in muscle, liver and kidney
- Estimated acute dietary exposure    The GEADE is 1.9 µg/day for the general population, which represents approximately 80% of the ARfD.  
  
The GEADE is 0.57 µg/day for children, which represents approximately 94% of the ARfD.

**Recommended maximum residue limits (MRLs)<sup>a</sup>**

Species	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	3.3	3.5	0.5

<sup>a</sup> There were insufficient zilpaterol residue data to adequately consider exposure to residues in lungs and other edible offal of cattle apart from liver and kidney.

# Annex 3

## Meeting agenda



Food and Agriculture  
Organization of the  
United Nations



World Health  
Organization

81<sup>st</sup> JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)  
FAO Headquarters, Rome 17-26 November 2015

**Opening:**  
Philippine Room (C 277), 17 November 2015, 9.30h

### Draft Agenda

1. Opening
2. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs
3. Adoption of Agenda
4. Declarations of Interests (information by the Secretariat on any declared interests and discussion)
5. Matters of interest arising from previous Sessions of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF)
  - a. Concern forms on Lasalocid sodium raised by CCRVDF
  - b. Request from CCRVDF 22 on MRLs for generic fish species
6. Other matters of interest arising from FAO and WHO
  - a. Update on new FAO JECFA database on residues of veterinary drugs
  - b. Update on FAO/WHO CIFOCoss database
7. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full Committee)
8. Evaluations

#### Veterinary drug residues

- Diflubenzuron
- Ethoxyquin

- Ivermectin
  - Lasalocid sodium (response to concern forms)
  - Sisapronil (formerly known as phenylpyrazole)
  - Teflubenzuron
  - Zilpaterol hydrochloride
9. General considerations
- JECFA response to CCRVDF 22 on MRLs for generic fish species
  - New JECFA guidance on residues of veterinary drugs
  - Acute Reference Dose (ARfD) for veterinary drugs
  - Issues arising from JMPR for consideration by JECFA
  - Approach for dietary exposure assessment of compounds used for multiple purposes (veterinary drug, pesticide)
  - Update on the revision of the Principles and Methods for the Risk Assessment of Chemicals in Food (EHC 240)
10. Other matters as may be brought forth by the Committee during discussions at the meeting.
11. Adoption of the report.

## SELECTED WHO PUBLICATIONS OF RELATED INTEREST

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### **Evaluation of Certain Food Additives and Contaminants**

Eightieth Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 995, 2016 (114 pages)

### **Safety Evaluation of Certain Food Additives and Contaminants**

Eightieth Meeting of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Food Additives Series, No. 71, 2015 (132 pages)

### **Evaluation of Certain Food Additives**

Seventy-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 990, 2015 (124 pages)

### **Safety Evaluation of Certain Food Additives**

Seventy-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 70, 2015 (369 pages)

### **Evaluation of Certain Veterinary Drug Residues in Food**

Seventy-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 988, 2014 (127 pages)

### **Toxicological Evaluation of Certain Veterinary Drug Residues in Food**

Seventy-eighth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 69, 2014 (241 pages)

### **Evaluation of Certain Food Additives and Contaminants**

Seventy-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 983, 2013 (75 pages)

### **Safety Evaluation of Certain Food Additives and Contaminants**

Seventy-seventh Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 68, 2013 (335 pages)

### **Evaluation of Certain Food Additives**

Seventy-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 974, 2012 (190 pages)

### **Safety Evaluation of Certain Food Additives**

Seventy-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 67, 2012 (335 pages)

## Evaluation of certain veterinary drug residues in food

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of residues of certain veterinary drugs in food and to recommend maximum levels for such residues in food.

The first part of the report considers general principles regarding the evaluation of residues of veterinary drugs within the terms of reference of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), including MRLs for generic fish species, acute reference doses (ARfDs) for veterinary drugs, an approach for dietary exposure assessment of compounds used for multiple purposes (i.e. veterinary drugs and pesticides), dietary exposure assessment for less-than-lifetime exposure, and the assessment of short-term (90-day and 12-month) studies in dogs.

Summaries follow of the Committee's evaluations of toxicological and residue data on a variety of veterinary drugs: two insecticides (diflubenzuron and teflubenzuron), an antiparasitic agent (ivermectin), an ectoparasiticide (sisapronil) and a  $\beta_2$ -adrenoceptor agonist (zilpaterol hydrochloride). In addition, the Committee considered issues raised in concern forms from the Codex Committee on Residues of Veterinary Drugs in Foods on lasalocid sodium, an antiparasitic agent. Annexed to the report is a summary of the Committee's recommendations on these drugs, including acceptable daily intakes (ADIs), ARfDs and proposed MRLs.

ISBN 978 92 4 120997 7



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